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(54) Title: G-PROTEIN COUPLED RECEPTORS

(57) Abstract: The invention provides human G-protein coupled receptors (GCREC) and polynucleotides which identify and encode GCREC. The invention also provides expression vectors, host cells, antibodies, agonist, and antagonist. The invention also provides methods for diagnosing, treating, or preventing disorders associated with aberrant expression of GCREC.

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## G-PROTEIN COUPLED RECEPTORS

### TECHNICAL FIELD

This invention relates to nucleic acid and amino acid sequences of G-protein coupled  
5 receptors and to the use of these sequences in the diagnosis, treatment, and prevention of cell  
proliferative, neurological, cardiovascular, gastrointestinal, autoimmune/inflammatory, and metabolic  
disorders, and viral infections, and in the assessment of the effects of exogenous compounds on the  
expression of nucleic acid and amino acid sequences of G-protein coupled receptors.

### BACKGROUND OF THE INVENTION

Signal transduction is the general process by which cells respond to extracellular signals.  
Signal transduction across the plasma membrane begins with the binding of a signal molecule, e.g., a  
hormone, neurotransmitter, or growth factor, to a cell membrane receptor. The receptor, thus  
activated, triggers an intracellular biochemical cascade that ends with the activation of an intracellular  
15 target molecule, such as a transcription factor. This process of signal transduction regulates all types  
of cell functions including cell proliferation, differentiation, and gene transcription. The G-protein  
coupled receptors (GPCRs), encoded by one of the largest families of genes yet identified, play a  
central role in the transduction of extracellular signals across the plasma membrane. GPCRs have a  
proven history of being successful therapeutic targets.

20 GPCRs are integral membrane proteins characterized by the presence of seven hydrophobic  
transmembrane domains which together form a bundle of antiparallel alpha ( $\alpha$ ) helices. GPCRs range  
in size from under 400 to over 1000 amino acids (Strosberg, A.D. (1991) Eur. J. Biochem. 196:1-10;  
Coughlin, S.R. (1994) Curr. Opin. Cell Biol. 6:191-197). The amino-terminus of a GPCR is  
extracellular, is of variable length, and is often glycosylated. The carboxy-terminus is cytoplasmic  
25 and generally phosphorylated. Extracellular loops alternate with intracellular loops and link the  
transmembrane domains. Cysteine disulfide bridges linking the second and third extracellular loops  
may interact with agonists and antagonists. The most conserved domains of GPCRs are the  
transmembrane domains and the first two cytoplasmic loops. The transmembrane domains account,  
in part, for structural and functional features of the receptor. In most cases, the bundle of  $\alpha$  helices  
30 forms a ligand-binding pocket. The extracellular N-terminal segment, or one or more of the three  
extracellular loops, may also participate in ligand binding. Ligand binding activates the receptor by  
inducing a conformational change in intracellular portions of the receptor. In turn, the large, third  
intracellular loop of the activated receptor interacts with a heterotrimeric guanine nucleotide binding  
(G) protein complex which mediates further intracellular signaling activities, including the activation  
35 of second messengers such as cyclic AMP (cAMP), phospholipase C, and inositol triphosphate, and

the interaction of the activated GPCR with ion channel proteins. (See, e.g., Watson, S. and S. Arkinstall (1994) The G-protein Linked Receptor Facts Book, Academic Press, San Diego CA, pp. 2-6; Bolander, F.F. (1994) Molecular Endocrinology, Academic Press, San Diego CA, pp. 162-176; Baldwin, J.M. (1994) *Curr. Opin. Cell Biol.* 6:180-190.)

5 GPCRs include receptors for sensory signal mediators (e.g., light and olfactory stimulatory molecules); adenosine,  $\gamma$ -aminobutyric acid (GABA), hepatocyte growth factor, melanocortins, neuropeptide Y, opioid peptides, opsins, somatostatin, tachykinins, vasoactive intestinal polypeptide family, and vasopressin; biogenic amines (e.g., dopamine, epinephrine and norepinephrine, histamine, glutamate (metabotropic effect), acetylcholine (muscarinic effect), and serotonin); chemokines; lipid  
10 mediators of inflammation (e.g., prostaglandins and prostanoids, platelet activating factor, and leukotrienes); and peptide hormones (e.g., bombesin, bradykinin, calcitonin, C5a anaphylatoxin, endothelin, follicle-stimulating hormone (FSH), gonadotropic-releasing hormone (GnRH), neurokinin, and thyrotropin-releasing hormone (TRH), and oxytocin). GPCRs which act as receptors for stimuli that have yet to be identified are known as orphan receptors.

15 The diversity of the GPCR family is further increased by alternative splicing. Many GPCR genes contain introns, and there are currently over 30 such receptors for which splice variants have been identified. The largest number of variations are at the protein C-terminus. N-terminal and cytoplasmic loop variants are also frequent, while variants in the extracellular loops or transmembrane domains are less common. Some receptors have more than one site at which variance  
20 can occur. The splicing variants appear to be functionally distinct, based upon observed differences in distribution, signaling, coupling, regulation, and ligand binding profiles (Kilpatrick, G.J. et al. (1999) *Trends Pharmacol. Sci.* 20:294-301).

GPCRs can be divided into three major subfamilies: the rhodopsin-like, secretin-like, and metabotropic glutamate receptor subfamilies. Members of these GPCR subfamilies share similar  
25 functions and the characteristic seven transmembrane structure, but have divergent amino acid sequences. The largest family consists of the rhodopsin-like GPCRs, which transmit diverse extracellular signals including hormones, neurotransmitters, and light. Rhodopsin is a photosensitive GPCR found in animal retinas. In vertebrates, rhodopsin molecules are embedded in membranous stacks found in photoreceptor (rod) cells. Each rhodopsin molecule responds to a photon of light by  
30 triggering a decrease in cGMP levels which leads to the closure of plasma membrane sodium channels. In this manner, a visual signal is converted to a neural impulse. Other rhodopsin-like GPCRs are directly involved in responding to neurotransmitters. These GPCRs include the receptors for adrenaline (adrenergic receptors), acetylcholine (muscarinic receptors), adenosine, galanin, and glutamate (N-methyl-D-aspartate/NMDA receptors). (Reviewed in Watson, S. and S. Arkinstall  
35 (1994) The G-Protein Linked Receptor Facts Book, Academic Press, San Diego CA, pp. 7-9, 19-22,

32-35, 130-131, 214-216, 221-222; Habert-Ortoli, E. et al. (1994) Proc. Natl. Acad. Sci. USA 91:9780-9783.)

The galanin receptors mediate the activity of the neuroendocrine peptide galanin, which inhibits secretion of insulin, acetylcholine, serotonin and noradrenaline, and stimulates prolactin and growth hormone release. Galanin receptors are involved in feeding disorders, pain, depression, and Alzheimer's disease (Kask, K. et al. (1997) Life Sci. 60:1523-1533). Other nervous system rhodopsin-like GPCRs include a growing family of receptors for lysophosphatidic acid and other lysophospholipids, which appear to have roles in development and neuropathology (Chun, J. et al. (1999) Cell Biochem. Biophys. 30:213-242).

10 The largest subfamily of GPCRs, the olfactory receptors, are also members of the rhodopsin-like GPCR family. These receptors function by transducing odorant signals. Numerous distinct olfactory receptors are required to distinguish different odors. Each olfactory sensory neuron expresses only one type of olfactory receptor, and distinct spatial zones of neurons expressing distinct receptors are found in nasal passages. For example, the RA1c receptor which was isolated from a rat brain library, has been shown to be limited in expression to very distinct regions of the brain and a defined zone of the olfactory epithelium (Raming, K. et al. (1998) Receptors Channels 6:141-151). However, the expression of olfactory-like receptors is not confined to olfactory tissues. For example, three rat genes encoding olfactory-like receptors having typical GPCR characteristics showed expression patterns not only in taste and olfactory tissue, but also in male reproductive tissue  
15 (Thomas, M.B. et al. (1996) Gene 178:1-5).

Members of the secretin-like GPCR subfamily have as their ligands peptide hormones such as secretin, calcitonin, glucagon, growth hormone-releasing hormone, parathyroid hormone, and vasoactive intestinal peptide. For example, the secretin receptor responds to secretin, a peptide hormone that stimulates the secretion of enzymes and ions in the pancreas and small intestine  
25 (Watson, supra, pp. 278-283). Secretin receptors are about 450 amino acids in length and are found in the plasma membrane of gastrointestinal cells. Binding of secretin to its receptor stimulates the production of cAMP.

Examples of secretin-like GPCRs implicated in inflammation and the immune response include the EGF module-containing, mucin-like hormone receptor (Emr1) and CD97 receptor  
30 proteins. These GPCRs are members of the recently characterized EGF-TM7 receptors subfamily. These seven transmembrane hormone receptors exist as heterodimers in vivo and contain between three and seven potential calcium-binding EGF-like motifs. CD97 is predominantly expressed in leukocytes and is markedly upregulated on activated B and T cells (McKnight, A.J. and S. Gordon (1998) J. Leukoc. Biol. 63:271-280).

35 The third GPCR subfamily is the metabotropic glutamate receptor family. Glutamate is the

major excitatory neurotransmitter in the central nervous system. The metabotropic glutamate receptors modulate the activity of intracellular effectors, and are involved in long-term potentiation (Watson, supra, p.130). The  $\text{Ca}^{2+}$ -sensing receptor, which senses changes in the extracellular concentration of calcium ions, has a large extracellular domain including clusters of acidic amino acids which may be involved in calcium binding. The metabotropic glutamate receptor family also includes pheromone receptors, the  $\text{GABA}_B$  receptors, and the taste receptors.

Other subfamilies of GPCRs include two groups of chemoreceptor genes found in the nematodes Caenorhabditis elegans and Caenorhabditis briggsae, which are distantly related to the mammalian olfactory receptor genes. The yeast pheromone receptors STE2 and STE3, involved in the response to mating factors on the cell membrane, have their own seven-transmembrane signature, as do the cAMP receptors from the slime mold Dictyostelium discoideum, which are thought to regulate the aggregation of individual cells and control the expression of numerous developmentally-regulated genes.

GPCR mutations, which may cause loss of function or constitutive activation, have been associated with numerous human diseases (Coughlin, supra). For instance, retinitis pigmentosa may arise from mutations in the rhodopsin gene. Furthermore, somatic activating mutations in the thyrotropin receptor have been reported to cause hyperfunctioning thyroid adenomas, suggesting that certain GPCRs susceptible to constitutive activation may behave as protooncogenes (Parma, J. et al. (1993) Nature 365:649-651). GPCR receptors for the following ligands also contain mutations associated with human disease: lutenizing hormone (precocious puberty); vasopressin  $\text{V}_2$  (X-linked nephrogenic diabetes); glucagon (diabetes and hypertension); calcium (hyperparathyroidism, hypocalcuria, hypercalcemia); parathyroid hormone (short limbed dwarfism);  $\beta_3$ -adrenoceptor (obesity, non-insulin-dependent diabetes mellitus); growth hormone releasing hormone (dwarfism); and adrenocorticotropin (glucocorticoid deficiency) (Wilson, S. et al. (1998) Br. J. Pharmacol. 125:1387-1392; Stadel, J.M. et al. (1997) Trends Pharmacol. Sci. 18:430-437). GPCRs are also involved in depression, schizophrenia, sleeplessness, hypertension, anxiety, stress, renal failure, and several cardiovascular disorders (Horn, F. and G. Vriend (1998) J. Mol. Med. 76:464-468).

In addition, within the past 20 years several hundred new drugs have been recognized that are directed towards activating or inhibiting GPCRs. The therapeutic targets of these drugs span a wide range of diseases and disorders, including cardiovascular, gastrointestinal, and central nervous system disorders as well as cancer, osteoporosis and endometriosis (Wilson, supra; Stadel, supra). For example, the dopamine agonist L-dopa is used to treat Parkinson's disease, while a dopamine antagonist is used to treat schizophrenia and the early stages of Huntington's disease. Agonists and antagonists of adrenoceptors have been used for the treatment of asthma, high blood pressure, other cardiovascular disorders, and anxiety; muscarinic agonists are used in the treatment of glaucoma and

tachycardia; serotonin 5HT<sub>1D</sub> antagonists are used against migraine; and histamine H<sub>1</sub> antagonists are used against allergic and anaphylactic reactions, hay fever, itching, and motion sickness (Horn, supra).

Recent research suggests potential future therapeutic uses for GPCRs in the treatment of metabolic disorders including diabetes, obesity, and osteoporosis. For example, mutant V2 vasopressin receptors causing nephrogenic diabetes could be functionally rescued in vitro by co-expression of a C-terminal V2 receptor peptide spanning the region containing the mutations. This result suggests a possible novel strategy for disease treatment (Schöneberg, T. et al. (1996) EMBO J. 15:1283-1291). Mutations in melanocortin-4 receptor (MC4R) are implicated in human weight regulation and obesity. As with the vasopressin V2 receptor mutants, these MC4R mutants are defective in trafficking to the plasma membrane (Ho, G. and R.G. MacKenzie (1999) J. Biol. Chem. 274:35816-35822), and thus might be treated with a similar strategy. The type 1 receptor for parathyroid hormone (PTH) is a GPCR that mediates the PTH-dependent regulation of calcium homeostasis in the bloodstream. Study of PTH/receptor interactions may enable the development of novel PTH receptor ligands for the treatment of osteoporosis (Mannstadt, M. et al. (1999) Am. J. Physiol. 277:F665-F675).

The chemokine receptor group of GPCRs have potential therapeutic utility in inflammation and infectious disease. (For review, see Locati, M. and P.M. Murphy (1999) Annu. Rev. Med. 50:425-440.) Chemokines are small polypeptides that act as intracellular signals in the regulation of leukocyte trafficking, hematopoiesis, and angiogenesis. Targeted disruption of various chemokine receptors in mice indicates that these receptors play roles in pathologic inflammation and in autoimmune disorders such as multiple sclerosis. Chemokine receptors are also exploited by infectious agents, including herpesviruses and the human immunodeficiency virus (HIV-1) to facilitate infection. A truncated version of chemokine receptor CCR5, which acts as a coreceptor for infection of T-cells by HIV-1, results in resistance to AIDS, suggesting that CCR5 antagonists could be useful in preventing the development of AIDS.

The discovery of new G-protein coupled receptors, and the polynucleotides encoding them, satisfies a need in the art by providing new compositions which are useful in the diagnosis, prevention, and treatment of cell proliferative, neurological, cardiovascular, gastrointestinal, autoimmune/inflammatory, and metabolic disorders, and viral infections, and in the assessment of the effects of exogenous compounds on the expression of nucleic acid and amino acid sequences of G-protein coupled receptors.

## SUMMARY OF THE INVENTION

The invention features purified polypeptides, G-protein coupled receptors, referred to

collectively as "GCREC" and individually as "GCREC-1," "GCREC-2," "GCREC-3," "GCREC-4," "GCREC-5," "GCREC-6," "GCREC-7," "GCREC-8," "GCREC-9," "GCREC-10," "GCREC-11," "GCREC-12," "GCREC-13," "GCREC-14," "GCREC-15," "GCREC-16," "GCREC-17," "GCREC-18," and "GCREC-19." In one aspect, the invention provides an isolated polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. In one alternative, the invention provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1-19.

The invention further provides an isolated polynucleotide encoding a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. In one alternative, the polynucleotide encodes a polypeptide selected from the group consisting of SEQ ID NO:1-19. In another alternative, the polynucleotide is selected from the group consisting of SEQ ID NO:20-38.

Additionally, the invention provides a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. In one alternative, the invention provides a cell transformed with the recombinant polynucleotide. In another alternative, the invention provides a transgenic organism comprising the recombinant polynucleotide.

The invention also provides a method for producing a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least

90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. The method comprises a)  
 5 culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide encoding the polypeptide, and b) recovering the polypeptide so expressed.

Additionally, the invention provides an isolated antibody which specifically binds to a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid  
 10 sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group  
 15 consisting of SEQ ID NO:1-19.

The invention further provides an isolated polynucleotide selected from the group consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID  
 20 NO:20-38, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). In one alternative, the polynucleotide comprises at least 60 contiguous nucleotides.

Additionally, the invention provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide selected from the group  
 25 consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). The  
 30 method comprises a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and b) detecting the presence or absence of said hybridization complex, and optionally, if  
 35 present, the amount thereof. In one alternative, the probe comprises at least 60 contiguous



nucleotides.

The invention further provides a method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide selected from the group consisting of a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of  
5 SEQ ID NO:20-38, b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, c) a polynucleotide complementary to the polynucleotide of a), d) a polynucleotide complementary to the polynucleotide of b), and e) an RNA equivalent of a)-d). The method comprises a) amplifying said target polynucleotide or fragment thereof using polymerase chain  
10 reaction amplification, and b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

The invention further provides a composition comprising an effective amount of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a  
15 naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and a pharmaceutically acceptable excipient. In one embodiment, the  
20 composition comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. The invention additionally provides a method of treating a disease or condition associated with decreased expression of functional GCREC, comprising administering to a patient in need of such treatment the composition.

The invention also provides a method for screening a compound for effectiveness as an  
25 agonist of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an  
30 immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. The method comprises a) exposing a sample comprising the polypeptide to a compound, and b) detecting agonist activity in the sample. In one alternative, the invention provides a composition comprising an agonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of  
35 treating a disease or condition associated with decreased expression of functional GCREC,

comprising administering to a patient in need of such treatment the composition.

Additionally, the invention provides a method for screening a compound for effectiveness as an antagonist of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide  
5 comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. The method comprises a) exposing a sample comprising the  
10 polypeptide to a compound, and b) detecting antagonist activity in the sample. In one alternative, the invention provides a composition comprising an antagonist compound identified by the method and a pharmaceutically acceptable excipient. In another alternative, the invention provides a method of treating a disease or condition associated with overexpression of functional GCREC, comprising administering to a patient in need of such treatment the composition.

15 The invention further provides a method of screening for a compound that specifically binds to a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide  
20 having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. The method comprises a) combining the polypeptide with at least one test compound under suitable conditions, and b) detecting binding of the polypeptide to the test compound, thereby identifying a compound that specifically binds to the polypeptide.

25 The invention further provides a method of screening for a compound that modulates the activity of a polypeptide selected from the group consisting of a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, c) a biologically active fragment of a polypeptide  
30 having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19. The method comprises a) combining the polypeptide with at least one test compound under conditions permissive for the activity of the polypeptide, b) assessing the activity of the polypeptide in the presence of the test compound, and c) comparing the activity of the

polypeptide in the presence of the test compound with the activity of the polypeptide in the absence of the test compound, wherein a change in the activity of the polypeptide in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide.

The invention further provides a method for screening a compound for effectiveness in  
 5 altering expression of a target polynucleotide, wherein said target polynucleotide comprises a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, the method comprising a) exposing a sample comprising the target polynucleotide to a compound, and b) detecting altered expression of the target polynucleotide.

The invention further provides a method for assessing toxicity of a test compound, said  
 10 method comprising a) treating a biological sample containing nucleic acids with the test compound; b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at least 20 contiguous nucleotides of a polynucleotide selected from the group consisting of i) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, ii) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a  
 15 polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, iii) a polynucleotide having a sequence complementary to i), iv) a polynucleotide complementary to the polynucleotide of ii), and v) an RNA equivalent of i)-iv). Hybridization occurs under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide selected from the group consisting of i) a  
 20 polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, ii) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38, iii) a polynucleotide complementary to the polynucleotide of i), iv) a polynucleotide complementary to the polynucleotide of ii), and v) an RNA equivalent of i)-iv). Alternatively, the target  
 25 polynucleotide comprises a fragment of a polynucleotide sequence selected from the group consisting of i)-v) above; c) quantifying the amount of hybridization complex; and d) comparing the amount of hybridization complex in the treated biological sample with the amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

30

### BRIEF DESCRIPTION OF THE TABLES

Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide sequences of the present invention.

Table 2 shows the GenBank identification number and annotation of the nearest GenBank homolog for polypeptides of the invention. The probability score for the match between each polypeptide and its GenBank homolog is also shown.

Table 3 shows structural features of polypeptide sequences of the invention, including  
5 predicted motifs and domains, along with the methods, algorithms, and searchable databases used for analysis of the polypeptides.

Table 4 lists the cDNA and/or genomic DNA fragments which were used to assemble polynucleotide sequences of the invention, along with selected fragments of the polynucleotide sequences.

10 Table 5 shows the representative cDNA library for polynucleotides of the invention.

Table 6 provides an appendix which describes the tissues and vectors used for construction of the cDNA libraries shown in Table 5.

Table 7 shows the tools, programs, and algorithms used to analyze the polynucleotides and polypeptides of the invention, along with applicable descriptions, references, and threshold  
15 parameters.

## DESCRIPTION OF THE INVENTION

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular machines, materials and methods described, as these  
20 may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a  
25 reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.  
30 Although any machines, materials, and methods similar or equivalent to those described herein can be used to practice or test the present invention, the preferred machines, materials and methods are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, protocols, reagents and vectors which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the  
35 invention is not entitled to antedate such disclosure by virtue of prior invention.

## DEFINITIONS

"GCREC" refers to the amino acid sequences of substantially purified GCREC obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and human, and from any source, whether natural, synthetic, semi-synthetic, or recombinant.

5       The term "agonist" refers to a molecule which intensifies or mimics the biological activity of GCREC. Agonists may include proteins, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of GCREC either by directly interacting with GCREC or by acting on components of the biological pathway in which GCREC participates.

      An "allelic variant" is an alternative form of the gene encoding GCREC. Allelic variants may  
10   result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. A gene may have none, one, or many allelic variants of its naturally occurring form. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times  
15   in a given sequence.

      "Altered" nucleic acid sequences encoding GCREC include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polypeptide the same as GCREC or a polypeptide with at least one functional characteristic of GCREC. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide  
20   probe of the polynucleotide encoding GCREC, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding GCREC. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent GCREC. Deliberate amino acid substitutions may be made on the basis of similarity in  
25   polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of GCREC is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, and positively charged amino acids may include lysine and arginine. Amino acids with uncharged polar side chains having similar hydrophilicity values may include: asparagine and glutamine; and serine and threonine.  
30   Amino acids with uncharged side chains having similar hydrophilicity values may include: leucine, isoleucine, and valine; glycine and alanine; and phenylalanine and tyrosine.

      The terms "amino acid" and "amino acid sequence" refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or synthetic molecules. Where "amino acid sequence" is recited to refer to a sequence of a naturally occurring  
35   protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid

sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification" relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art.

5       The term "antagonist" refers to a molecule which inhibits or attenuates the biological activity of GCREC. Antagonists may include proteins such as antibodies, nucleic acids, carbohydrates, small molecules, or any other compound or composition which modulates the activity of GCREC either by directly interacting with GCREC or by acting on components of the biological pathway in which GCREC participates.

10       The term "antibody" refers to intact immunoglobulin molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding an epitopic determinant. Antibodies that bind GCREC polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the  
15 translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

      The term "antigenic determinant" refers to that region of a molecule (i.e., an epitope) that  
20 makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (particular regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

25       The term "antisense" refers to any composition capable of base-pairing with the "sense" (coding) strand of a specific nucleic acid sequence. Antisense compositions may include DNA; RNA; peptide nucleic acid (PNA); oligonucleotides having modified backbone linkages such as phosphorothioates, methylphosphonates, or benzylphosphonates; oligonucleotides having modified sugar groups such as 2'-methoxyethyl sugars or 2'-methoxyethoxy sugars; or oligonucleotides having  
30 modified bases such as 5-methyl cytosine, 2'-deoxyuracil, or 7-deaza-2'-deoxyguanosine. Antisense molecules may be produced by any method including chemical synthesis or transcription. Once introduced into a cell, the complementary antisense molecule base-pairs with a naturally occurring nucleic acid sequence produced by the cell to form duplexes which block either transcription or translation. The designation "negative" or "minus" can refer to the antisense strand, and the  
35 designation "positive" or "plus" can refer to the sense strand of a reference DNA molecule.

The term "biologically active" refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" or "immunogenic" refers to the capability of the natural, recombinant, or synthetic GCREC, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific  
 5 antibodies.

"Complementary" describes the relationship between two single-stranded nucleic acid sequences that anneal by base-pairing. For example, 5'-AGT-3' pairs with its complement, 3'-TCA-5'.

A "composition comprising a given polynucleotide sequence" and a "composition comprising  
 10 a given amino acid sequence" refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation or an aqueous solution. Compositions comprising polynucleotide sequences encoding GCREC or fragments of GCREC may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be  
 15 deployed in an aqueous solution containing salts (e.g., NaCl), detergents (e.g., sodium dodecyl sulfate; SDS), and other components (e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.).

"Consensus sequence" refers to a nucleic acid sequence which has been subjected to repeated DNA sequence analysis to resolve uncalled bases, extended using the XL-PCR kit (Applied Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been  
 20 assembled from one or more overlapping cDNA, EST, or genomic DNA fragments using a computer program for fragment assembly, such as the GELVIEW fragment assembly system (GCG, Madison WI) or Phrap (University of Washington, Seattle WA). Some sequences have been both extended and assembled to produce the consensus sequence.

"Conservative amino acid substitutions" are those substitutions that are predicted to least  
 25 interfere with the properties of the original protein, i.e., the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. The table below shows amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative amino acid substitutions.

	Original Residue	Conservative Substitution
30	Ala	Gly, Ser
	Arg	His, Lys
	Asn	Asp, Gln, His
	Asp	Asn, Glu
	Cys	Ala, Ser
35	Gln	Asn, Glu, His
	Glu	Asp, Gln, His
	Gly	Ala

	His	Asn, Arg, Gln, Glu
	Ile	Leu, Val
	Leu	Ile, Val
5	Lys	Arg, Gln, Glu
	Met	Leu, Ile
	Phe	His, Met, Leu, Trp, Tyr
	Ser	Cys, Thr
	Thr	Ser, Val
	Trp	Phe, Tyr
10	Tyr	His, Phe, Trp
	Val	Ile, Leu, Thr

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Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

A "deletion" refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term "derivative" refers to a chemically modified polynucleotide or polypeptide. Chemical modifications of a polynucleotide can include, for example, replacement of hydrogen by an alkyl, acyl, hydroxyl, or amino group. A derivative polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

A "detectable label" refers to a reporter molecule or enzyme that is capable of generating a measurable signal and is covalently or noncovalently joined to a polynucleotide or polypeptide.

"Differential expression" refers to increased or upregulated; or decreased, downregulated, or absent gene or protein expression, determined by comparing at least two different samples. Such comparisons may be carried out between, for example, a treated and an untreated sample, or a diseased and a normal sample.

"Exon shuffling" refers to the recombination of different coding regions (exons). Since an exon may represent a structural or functional domain of the encoded protein, new proteins may be assembled through the novel reassortment of stable substructures, thus allowing acceleration of the evolution of new protein functions.

A "fragment" is a unique portion of GCREC or the polynucleotide encoding GCREC which is identical in sequence to but shorter in length than the parent sequence. A fragment may comprise up to the entire length of the defined sequence, minus one nucleotide/amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous nucleotides or amino acid residues. A



fragment used as a probe, primer, antigen, therapeutic molecule, or for other purposes, may be at least 5, 10, 15, 16, 20, 25, 30, 40, 50, 60, 75, 100, 150, 250 or at least 500 contiguous nucleotides or amino acid residues in length. Fragments may be preferentially selected from certain regions of a molecule.

For example, a polypeptide fragment may comprise a certain length of contiguous amino acids

- 5 selected from the first 250 or 500 amino acids (or first 25% or 50%) of a polypeptide as shown in a certain defined sequence. Clearly these lengths are exemplary, and any length that is supported by the specification, including the Sequence Listing, tables, and figures, may be encompassed by the present embodiments.

- A fragment of SEQ ID NO:20-38 comprises a region of unique polynucleotide sequence that  
10 specifically identifies SEQ ID NO:20-38, for example, as distinct from any other sequence in the genome from which the fragment was obtained. A fragment of SEQ ID NO:20-38 is useful, for example, in hybridization and amplification technologies and in analogous methods that distinguish SEQ ID NO:20-38 from related polynucleotide sequences. The precise length of a fragment of SEQ ID NO:20-38 and the region of SEQ ID NO:20-38 to which the fragment corresponds are routinely  
15 determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

- A fragment of SEQ ID NO:1-19 is encoded by a fragment of SEQ ID NO:20-38. A fragment of SEQ ID NO:1-19 comprises a region of unique amino acid sequence that specifically identifies SEQ ID NO:1-19. For example, a fragment of SEQ ID NO:1-19 is useful as an immunogenic peptide for the development of antibodies that specifically recognize SEQ ID NO:1-19. The precise length of  
20 a fragment of SEQ ID NO:1-19 and the region of SEQ ID NO:1-19 to which the fragment corresponds are routinely determinable by one of ordinary skill in the art based on the intended purpose for the fragment.

- A "full length" polynucleotide sequence is one containing at least a translation initiation codon (e.g., methionine) followed by an open reading frame and a translation termination codon. A  
25 "full length" polynucleotide sequence encodes a "full length" polypeptide sequence.

"Homology" refers to sequence similarity or, interchangeably, sequence identity, between two or more polynucleotide sequences or two or more polypeptide sequences.

- The terms "percent identity" and "% identity," as applied to polynucleotide sequences, refer to the percentage of residue matches between at least two polynucleotide sequences aligned using a  
30 standardized algorithm. Such an algorithm may insert, in a standardized and reproducible way, gaps in the sequences being compared in order to optimize alignment between two sequences, and therefore achieve a more meaningful comparison of the two sequences.

- Percent identity between polynucleotide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e  
35 sequence alignment program. This program is part of the LASERGENE software package, a suite of

molecular biological analysis programs (DNASTAR, Madison WI). CLUSTAL V is described in Higgins, D.G. and P.M. Sharp (1989) CABIOS 5:151-153 and in Higgins, D.G. et al. (1992) CABIOS 8:189-191. For pairwise alignments of polynucleotide sequences, the default parameters are set as follows: Ktuple=2, gap penalty=5, window=4, and "diagonals saved"=4. The "weighted" residue weight table is selected as the default. Percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polynucleotide sequences.

Alternatively, a suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) (Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410), which is available from several sources, including the NCBI, Bethesda, MD, and on the Internet at <http://www.ncbi.nlm.nih.gov/BLAST/>. The BLAST software suite includes various sequence analysis programs including "blastn," that is used to align a known polynucleotide sequence with other polynucleotide sequences from a variety of databases. Also available is a tool called "BLAST 2 Sequences" that is used for direct pairwise comparison of two nucleotide sequences. "BLAST 2 Sequences" can be accessed and used interactively at <http://www.ncbi.nlm.nih.gov/gorf/bl2.html>. The "BLAST 2 Sequences" tool can be used for both blastn and blastp (discussed below). BLAST programs are commonly used with gap and other parameters set to default settings. For example, to compare two nucleotide sequences, one may use blastn with the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) set at default parameters. Such default parameters may be, for example:

20     *Matrix: BLOSUM62*  
        *Reward for match: 1*  
        *Penalty for mismatch: -2*  
        *Open Gap: 5 and Extension Gap: 2 penalties*  
        *Gap x drop-off: 50*  
 25     *Expect: 10*  
        *Word Size: 11*  
        *Filter: on*

Percent identity may be measured over the length of an entire defined sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined sequence, for instance, a fragment of at least 20, at least 30, at least 40, at least 50, at least 70, at least 100, or at least 200 contiguous nucleotides. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures, or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

35     Nucleic acid sequences that do not show a high degree of identity may nevertheless encode

similar amino acid sequences due to the degeneracy of the genetic code. It is understood that changes in a nucleic acid sequence can be made using this degeneracy to produce multiple nucleic acid sequences that all encode substantially the same protein.

The phrases "percent identity" and "% identity," as applied to polypeptide sequences, refer to the percentage of residue matches between at least two polypeptide sequences aligned using a standardized algorithm. Methods of polypeptide sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail above, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide.

Percent identity between polypeptide sequences may be determined using the default parameters of the CLUSTAL V algorithm as incorporated into the MEGALIGN version 3.12e sequence alignment program (described and referenced above). For pairwise alignments of polypeptide sequences using CLUSTAL V, the default parameters are set as follows: Ktuple=1, gap penalty=3, window=5, and "diagonals saved"=5. The PAM250 matrix is selected as the default residue weight table. As with polynucleotide alignments, the percent identity is reported by CLUSTAL V as the "percent similarity" between aligned polypeptide sequence pairs.

Alternatively the NCBI BLAST software suite may be used. For example, for a pairwise comparison of two polypeptide sequences, one may use the "BLAST 2 Sequences" tool Version 2.0.12 (April-21-2000) with blastp set at default parameters. Such default parameters may be, for example:

*Matrix: BLOSUM62*

*Open Gap: 11 and Extension Gap: 1 penalties*

*Gap x drop-off: 50*

*Expect: 10*

*Word Size: 3*

*Filter: on*

Percent identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

"Human artificial chromosomes" (HACs) are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size and which contain all of the elements required for

chromosome replication, segregation and maintenance.

The term "humanized antibody" refers to an antibody molecule in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

- 5 "Hybridization" refers to the process by which a polynucleotide strand anneals with a complementary strand through base pairing under defined hybridization conditions. Specific hybridization is an indication that two nucleic acid sequences share a high degree of complementarity. Specific hybridization complexes form under permissive annealing conditions and remain hybridized after the "washing" step(s). The washing step(s) is particularly important in determining the
- 10 stringency of the hybridization process, with more stringent conditions allowing less non-specific binding, i.e., binding between pairs of nucleic acid strands that are not perfectly matched. Permissive conditions for annealing of nucleic acid sequences are routinely determinable by one of ordinary skill in the art and may be consistent among hybridization experiments, whereas wash conditions may be varied among experiments to achieve the desired stringency, and therefore hybridization specificity.
- 15 Permissive annealing conditions occur, for example, at 68°C in the presence of about 6 x SSC, about 1% (w/v) SDS, and about 100 µg/ml sheared, denatured salmon sperm DNA.

Generally, stringency of hybridization is expressed, in part, with reference to the temperature under which the wash step is carried out. Such wash temperatures are typically selected to be about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of

20 the target sequence hybridizes to a perfectly matched probe. An equation for calculating  $T_m$  and conditions for nucleic acid hybridization are well known and can be found in Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; specifically see volume 2, chapter 9.

- 25 High stringency conditions for hybridization between polynucleotides of the present invention include wash conditions of 68°C in the presence of about 0.2 x SSC and about 0.1% SDS, for 1 hour. Alternatively, temperatures of about 65°C, 60°C, 55°C, or 42°C may be used. SSC concentration may be varied from about 0.1 to 2 x SSC, with SDS being present at about 0.1%. Typically, blocking reagents are used to block non-specific hybridization. Such blocking reagents
- 30 include, for instance, sheared and denatured salmon sperm DNA at about 100-200 µg/ml. Organic solvent, such as formamide at a concentration of about 35-50% v/v, may also be used under particular circumstances, such as for RNA:DNA hybridizations. Useful variations on these wash conditions will be readily apparent to those of ordinary skill in the art. Hybridization, particularly under high stringency conditions, may be suggestive of evolutionary similarity between the nucleotides. Such
- 35 similarity is strongly indicative of a similar role for the nucleotides and their encoded polypeptides.

The term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C<sub>0</sub>t or R<sub>0</sub>t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" and "addition" refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

An "immunogenic fragment" is a polypeptide or oligopeptide fragment of GCREC which is capable of eliciting an immune response when introduced into a living organism, for example, a mammal. The term "immunogenic fragment" also includes any polypeptide or oligopeptide fragment of GCREC which is useful in any of the antibody production methods disclosed herein or known in the art.

The term "microarray" refers to an arrangement of a plurality of polynucleotides, polypeptides, or other chemical compounds on a substrate.

The terms "element" and "array element" refer to a polynucleotide, polypeptide, or other chemical compound having a unique and defined position on a microarray.

The term "modulate" refers to a change in the activity of GCREC. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of GCREC.

The phrases "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material.

"Operably linked" refers to the situation in which a first nucleic acid sequence is placed in a functional relationship with a second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Operably linked DNA sequences may be in close proximity or contiguous and, where necessary to join two protein coding regions, in the same reading frame.

"Peptide nucleic acid" (PNA) refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of

amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell.

“Post-translational modification” of an GCREC may involve lipidation, glycosylation, phosphorylation, acetylation, racemization, proteolytic cleavage, and other modifications known in the art. These processes may occur synthetically or biochemically. Biochemical modifications will vary by cell type depending on the enzymatic milieu of GCREC.

“Probe” refers to nucleic acid sequences encoding GCREC, their complements, or fragments thereof, which are used to detect identical, allelic or related nucleic acid sequences. Probes are isolated oligonucleotides or polynucleotides attached to a detectable label or reporter molecule. Typical labels include radioactive isotopes, ligands, chemiluminescent agents, and enzymes.

“Primers” are short nucleic acids, usually DNA oligonucleotides, which may be annealed to a target polynucleotide by complementary base-pairing. The primer may then be extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification (and identification) of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCR).

Probes and primers as used in the present invention typically comprise at least 15 contiguous nucleotides of a known sequence. In order to enhance specificity, longer probes and primers may also be employed, such as probes and primers that comprise at least 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, or at least 150 consecutive nucleotides of the disclosed nucleic acid sequences. Probes and primers may be considerably longer than these examples, and it is understood that any length supported by the specification, including the tables, figures, and Sequence Listing, may be used.

Methods for preparing and using probes and primers are described in the references, for example Sambrook, J. et al. (1989) Molecular Cloning: A Laboratory Manual, 2<sup>nd</sup> ed., vol. 1-3, Cold Spring Harbor Press, Plainview NY; Ausubel, F.M. et al. (1987) Current Protocols in Molecular Biology, Greene Publ. Assoc. & Wiley-Intersciences, New York NY; Innis, M. et al. (1990) PCR Protocols, A Guide to Methods and Applications, Academic Press, San Diego CA. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge MA).

Oligonucleotides for use as primers are selected using software known in the art for such purpose. For example, OLIGO 4.06 software is useful for the selection of PCR primer pairs of up to 100 nucleotides each, and for the analysis of oligonucleotides and larger polynucleotides of up to 5,000 nucleotides from an input polynucleotide sequence of up to 32 kilobases. Similar primer selection programs have incorporated additional features for expanded capabilities. For example, the PrimOU primer selection program (available to the public from the Genome Center at University of

Texas South West Medical Center, Dallas TX) is capable of choosing specific primers from megabase sequences and is thus useful for designing primers on a genome-wide scope. The Primer3 primer selection program (available to the public from the Whitehead Institute/MIT Center for Genome Research, Cambridge MA) allows the user to input a "mispriming library," in which  
5 sequences to avoid as primer binding sites are user-specified. Primer3 is useful, in particular, for the selection of oligonucleotides for microarrays. (The source code for the latter two primer selection programs may also be obtained from their respective sources and modified to meet the user's specific needs.) The PrimeGen program (available to the public from the UK Human Genome Mapping Project Resource Centre, Cambridge UK) designs primers based on multiple sequence alignments,  
10 thereby allowing selection of primers that hybridize to either the most conserved or least conserved regions of aligned nucleic acid sequences. Hence, this program is useful for identification of both unique and conserved oligonucleotides and polynucleotide fragments. The oligonucleotides and polynucleotide fragments identified by any of the above selection methods are useful in hybridization technologies, for example, as PCR or sequencing primers, microarray elements, or specific probes to  
15 identify fully or partially complementary polynucleotides in a sample of nucleic acids. Methods of oligonucleotide selection are not limited to those described above.

A "recombinant nucleic acid" is a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two or more otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the  
20 artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques such as those described in Sambrook, supra. The term recombinant includes nucleic acids that have been altered solely by addition, substitution, or deletion of a portion of the nucleic acid. Frequently, a recombinant nucleic acid may include a nucleic acid sequence operably linked to a promoter sequence. Such a recombinant nucleic acid may be part of a vector that is used, for example, to  
25 transform a cell.

Alternatively, such recombinant nucleic acids may be part of a viral vector, e.g., based on a vaccinia virus, that could be use to vaccinate a mammal wherein the recombinant nucleic acid is expressed, inducing a protective immunological response in the mammal.

A "regulatory element" refers to a nucleic acid sequence usually derived from untranslated  
30 regions of a gene and includes enhancers, promoters, introns, and 5' and 3' untranslated regions (UTRs). Regulatory elements interact with host or viral proteins which control transcription, translation, or RNA stability.

"Reporter molecules" are chemical or biochemical moieties used for labeling a nucleic acid, amino acid, or antibody. Reporter molecules include radionuclides; enzymes; fluorescent,  
35 chemiluminescent, or chromogenic agents; substrates; cofactors; inhibitors; magnetic particles; and

other moieties known in the art.

An "RNA equivalent," in reference to a DNA sequence, is composed of the same linear sequence of nucleotides as the reference DNA sequence with the exception that all occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

The term "sample" is used in its broadest sense. A sample suspected of containing GCREC, nucleic acids encoding GCREC, or fragments thereof may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a substrate; a tissue; a tissue print; etc.

The terms "specific binding" and "specifically binding" refer to that interaction between a protein or peptide and an agonist, an antibody, an antagonist, a small molecule, or any natural or synthetic binding composition. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope "A," the presence of a polypeptide comprising the epitope A, or the presence of free unlabeled A, in a reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

The term "substantially purified" refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least 60% free, preferably at least 75% free, and most preferably at least 90% free from other components with which they are naturally associated.

A "substitution" refers to the replacement of one or more amino acid residues or nucleotides by different amino acid residues or nucleotides, respectively.

"Substrate" refers to any suitable rigid or semi-rigid support including membranes, filters, chips, slides, wafers, fibers, magnetic or nonmagnetic beads, gels, tubing, plates, polymers, microparticles and capillaries. The substrate can have a variety of surface forms, such as wells, trenches, pins, channels and pores, to which polynucleotides or polypeptides are bound.

A "transcript image" refers to the collective pattern of gene expression by a particular cell type or tissue under given conditions at a given time.

"Transformation" describes a process by which exogenous DNA is introduced into a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. The term "transformed cells" includes stably transformed cells in which the inserted DNA is capable of



replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "transgenic organism," as used herein, is any organism, including but not limited to animals and plants, in which one or more of the cells of the organism contains heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell, directly or indirectly by introduction into a precursor of the cell, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or in vitro fertilization, but rather is directed to the introduction of a recombinant DNA molecule. The transgenic organisms contemplated in accordance with the present invention include bacteria, cyanobacteria, fungi, plants and animals. The isolated DNA of the present invention can be introduced into the host by methods known in the art, for example infection, transfection, transformation or transconjugation. Techniques for transferring the DNA of the present invention into such organisms are widely known and provided in references such as Sambrook et al. (1989), supra.

A "variant" of a particular nucleic acid sequence is defined as a nucleic acid sequence having at least 40% sequence identity to the particular nucleic acid sequence over a certain length of one of the nucleic acid sequences using blastn with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of nucleic acids may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length. A variant may be described as, for example, an "allelic" (as defined above), "splice," "species," or "polymorphic" variant. A splice variant may have significant identity to a reference molecule, but will generally have a greater or lesser number of polynucleotides due to alternate splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or lack domains that are present in the reference molecule. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides will generally have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs) in which the polynucleotide sequence varies by one nucleotide base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

A "variant" of a particular polypeptide sequence is defined as a polypeptide sequence having at least 40% sequence identity to the particular polypeptide sequence over a certain length of one of

the polypeptide sequences using blastp with the "BLAST 2 Sequences" tool Version 2.0.9 (May-07-1999) set at default parameters. Such a pair of polypeptides may show, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% or greater sequence identity over a certain defined length of one of the polypeptides.

## THE INVENTION

The invention is based on the discovery of new human G-protein coupled receptors (GCREC), the polynucleotides encoding GCREC, and the use of these compositions for the diagnosis, treatment, or prevention of cell proliferative, neurological, cardiovascular, gastrointestinal, autoimmune/inflammatory, and metabolic disorders, and viral infections.

Table 1 summarizes the nomenclature for the full length polynucleotide and polypeptide sequences of the invention. Each polynucleotide and its corresponding polypeptide are correlated to a single Incyte project identification number (Incyte Project ID). Each polypeptide sequence is denoted by both a polypeptide sequence identification number (Polypeptide SEQ ID NO:) and an Incyte polypeptide sequence number (Incyte Polypeptide ID) as shown. Each polynucleotide sequence is denoted by both a polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and an Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) as shown.

Table 2 shows sequences with homology to the polypeptides of the invention as identified by BLAST analysis against the GenBank protein (genpept) database. Columns 1 and 2 show the polypeptide sequence identification number (Polypeptide SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for polypeptides of the invention. Column 3 shows the GenBank identification number (Genbank ID NO:) of the nearest GenBank homolog. Column 4 shows the probability score for the match between each polypeptide and its GenBank homolog. Column 5 shows the annotation of the GenBank homolog.

Table 3 shows various structural features of the polypeptides of the invention. Columns 1 and 2 show the polypeptide sequence identification number (SEQ ID NO:) and the corresponding Incyte polypeptide sequence number (Incyte Polypeptide ID) for each polypeptide of the invention. Column 3 shows the number of amino acid residues in each polypeptide. Column 4 shows potential phosphorylation sites, and column 5 shows potential glycosylation sites, as determined by the MOTIFS program of the GCG sequence analysis software package (Genetics Computer Group, Madison WI). Column 6 shows amino acid residues comprising signature sequences, domains, and motifs. Column 7 shows analytical methods for protein structure/function analysis and in some cases, searchable databases to which the analytical methods were applied.

Together, Tables 2 and 3 summarize the properties of each polypeptide of the invention, and

these properties establish that the claimed polypeptides are G-protein coupled receptors. For example, SEQ ID NO:1 is 40% identical to Meleagris gallopavo G protein-coupled P2Y nucleotide receptor (GenBank ID g2707256) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is  $4.0e-62$ , which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:1 also contains a rhodopsin family 7 transmembrane receptor domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS and BLAST analyses provide further corroborative evidence that SEQ ID NO:1 is G-protein coupled receptor. SEQ ID NO:2 was analyzed and annotated in a similar manner. These analyses indicate that SEQ ID NO:2 is a pheromone receptor (Dulac, C. and R. Axel (1995) Cell 83:195-206).

As a further example, SEQ ID NO:6 is 29% identical to human C-C chemokine receptor type 1 (GenBank ID g179985) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is  $1.6e-15$ , which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:6 also contains a 7 transmembrane receptor (rhodopsin family) domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:6 is a chemokine receptor.

As a further example, SEQ ID NO:9 is 95% identical to rat calcium-independent alpha-latrotoxin receptor (GenBank ID g3882981) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is 0.0, which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:9 also contains a 7-transmembrane receptor (secretin family) domain and a latrophilin/CL-1-like GPS domain, as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:9 is a latrophilin-related G-protein coupled receptor.

As a further example, SEQ ID NO:12 is 84% identical to Mus musculus G-protein coupled receptor GPR73 (GenBank ID g7248884) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is  $6.7e-166$ , which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:12 also contains a 7 transmembrane receptor (rhodopsin family) domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS analysis reveals the presence of a rhodopsin-like

GPCR superfamily signature (See Table 3). Additional data from MOTIFS and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:12 is a G-protein coupled receptor.

As a further example, SEQ ID NO:15 is 80% identical to rat serotonin receptor (GenBank ID g310075) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is  $2.5e-152$ , which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:15 also contains a rhodopsin family receptor domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, analyses provide further corroborative evidence that SEQ ID NO:15 is a G-protein coupled receptor.

As a further example, SEQ ID NO:16 is 71% identical to mouse olfactory receptor E3 (GenBank ID g3983382) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is  $1.9e-88$ , which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:16 also contains a rhodopsin family 7-transmembrane receptor domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:16 is an olfactory G-protein coupled receptor.

As a further example, SEQ ID NO:17 is 83% identical to mouse olfactory G-protein coupled receptor G3 (GenBank ID g3983398) as determined by the Basic Local Alignment Search Tool (BLAST). (See Table 2.) The BLAST probability score is  $5.0e-99$ , which indicates the probability of obtaining the observed polypeptide sequence alignment by chance. SEQ ID NO:17 also contains a rhodopsin family 7-transmembrane receptor domain as determined by searching for statistically significant matches in the hidden Markov model (HMM)-based PFAM database of conserved protein family domains. (See Table 3.) Data from BLIMPS, MOTIFS, and PROFILESCAN analyses provide further corroborative evidence that SEQ ID NO:17 is an olfactory G-protein coupled receptor. SEQ ID NO:2-5, SEQ ID NO:7-8, SEQ ID NO:10-11, SEQ ID NO:13-14, and SEQ ID NO:18-19 were analyzed and annotated in a similar manner. The algorithms and parameters for the analysis of SEQ ID NO:1-19 are described in Table 7.

As shown in Table 4, the full length polynucleotide sequences of the present invention were assembled using cDNA sequences or coding (exon) sequences derived from genomic DNA, or any combination of these two types of sequences. Columns 1 and 2 list the polynucleotide sequence identification number (Polynucleotide SEQ ID NO:) and the corresponding Incyte polynucleotide consensus sequence number (Incyte Polynucleotide ID) for each polynucleotide of the invention. Column 3 shows the length of each polynucleotide sequence in basepairs. Column 4 lists fragments

of the polynucleotide sequences which are useful, for example, in hybridization or amplification technologies that identify SEQ ID NO:20-38 or that distinguish between SEQ ID NO:20-38 and related polynucleotide sequences. Column 5 shows identification numbers corresponding to cDNA sequences, coding sequences (exons) predicted from genomic DNA, and/or sequence assemblages comprised of both cDNA and genomic DNA. These sequences were used to assemble the full length polynucleotide sequences of the invention. Columns 6 and 7 of Table 4 show the nucleotide start (5') and stop (3') positions of the cDNA and/or genomic sequences in column 5 relative to their respective full length sequences.

The identification numbers in Column 5 of Table 4 may refer specifically, for example, to Incyte cDNAs along with their corresponding cDNA libraries. For example, 7075196H1 is the identification number of an Incyte cDNA sequence, and BRAUTDR04 is the cDNA library from which it is derived. Incyte cDNAs for which cDNA libraries are not indicated were derived from pooled cDNA libraries (e.g., 71906055V1). Alternatively, the identification numbers in column 5 may refer to GenBank cDNAs or ESTs (e.g., g900324) which contributed to the assembly of the full length polynucleotide sequences. In addition, the identification numbers in column 5 may identify sequences derived from the ENSEMBL (The Sanger Centre, Cambridge, UK) database (*i.e.*, those sequences including the designation "ENST"). Alternatively, the identification numbers in column 5 may be derived from the NCBI RefSeq Nucleotide Sequence Records Database (*i.e.*, those sequences including the designation "NM" or "NT") or the NCBI RefSeq Protein Sequence Records (*i.e.*, those sequences including the designation "NP"). Alternatively, the identification numbers in column 5 may refer to assemblages of both cDNA and Genscan-predicted exons brought together by an "exon stitching" algorithm. For example, FL\_XXXXXX\_N<sub>1</sub>\_N<sub>2</sub>\_YYYYY\_N<sub>3</sub>\_N<sub>4</sub> represents a "stitched" sequence in which XXXXXX is the identification number of the cluster of sequences to which the algorithm was applied, and YYYYY is the number of the prediction generated by the algorithm, and N<sub>1,2,3...</sub>, if present, represent specific exons that may have been manually edited during analysis (See Example V). Alternatively, the identification numbers in column 5 may refer to assemblages of exons brought together by an "exon-stretching" algorithm. For example, FLXXXXXX\_gAAAAA\_gBBBBB\_1\_N is the identification number of a "stretched" sequence, with XXXXXX being the Incyte project identification number, gAAAAA being the GenBank identification number of the human genomic sequence to which the "exon-stretching" algorithm was applied, gBBBBB being the GenBank identification number or NCBI RefSeq identification number of the nearest GenBank protein homolog, and N referring to specific exons (See Example V). In instances where a RefSeq sequence was used as a protein homolog for the "exon-stretching" algorithm, a RefSeq identifier (denoted by "NM," "NP," or "NT") may be used in place of the GenBank identifier (*i.e.*, gBBBBB).

Alternatively, a prefix identifies component sequences that were hand-edited, predicted from genomic DNA sequences, or derived from a combination of sequence analysis methods. The following Table lists examples of component sequence prefixes and corresponding sequence analysis methods associated with the prefixes (see Example IV and Example V).

5	<b>Prefix</b>	<b>Type of analysis and/or examples of programs</b>
	GNN, GFG, ENST	Exon prediction from genomic sequences using, for example, GENSCAN (Stanford University, CA, USA) or FGENES (Computer Genomics Group, The Sanger Centre, Cambridge, UK).
	GBI	Hand-edited analysis of genomic sequences.
	FL	Stitched or stretched genomic sequences (see Example V).
10	INCY	Full length transcript and exon prediction from mapping of EST sequences to the genome. Genomic location and EST composition data are combined to predict the exons and resulting transcript.

In some cases, Incyte cDNA coverage redundant with the sequence coverage shown in column 5 was obtained to confirm the final consensus polynucleotide sequence, but the relevant Incyte cDNA identification numbers are not shown.

15 Table 5 shows the representative cDNA libraries for those full length polynucleotide sequences which were assembled using Incyte cDNA sequences. The representative cDNA library is the Incyte cDNA library which is most frequently represented by the Incyte cDNA sequences which were used to assemble and confirm the above polynucleotide sequences. The tissues and vectors which were used to construct the cDNA libraries shown in Table 5 are described in Table 6.

20 The invention also encompasses GCREC variants. A preferred GCREC variant is one which has at least about 80%, or alternatively at least about 90%, or even at least about 95% amino acid sequence identity to the GCREC amino acid sequence, and which contains at least one functional or structural characteristic of GCREC.

The invention also encompasses polynucleotides which encode GCREC. In a particular 25 embodiment, the invention encompasses a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:20-38, which encodes GCREC. The polynucleotide sequences of SEQ ID NO:20-38, as presented in the Sequence Listing, embrace the equivalent RNA sequences, wherein occurrences of the nitrogenous base thymine are replaced with uracil, and the sugar backbone is composed of ribose instead of deoxyribose.

30 The invention also encompasses a variant of a polynucleotide sequence encoding GCREC. In particular, such a variant polynucleotide sequence will have at least about 70%, or alternatively at

least about 85%, or even at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding GCREC. A particular aspect of the invention encompasses a variant of a polynucleotide sequence comprising a sequence selected from the group consisting of SEQ ID NO:20-38 which has at least about 70%, or alternatively at least about 85%, or even at least about 5 95% polynucleotide sequence identity to a nucleic acid sequence selected from the group consisting of SEQ ID NO:20-38. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of GCREC.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding GCREC, some bearing minimal 10 similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring GCREC, and all such variations are to be considered 15 as being specifically disclosed.

Although nucleotide sequences which encode GCREC and its variants are generally capable of hybridizing to the nucleotide sequence of the naturally occurring GCREC under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding GCREC or its derivatives possessing a substantially different codon usage, e.g., inclusion of non- 20 naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding GCREC and its derivatives without altering the encoded amino acid sequences include the production of RNA transcripts having more desirable properties, such as a 25 greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode GCREC and GCREC derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to 30 introduce mutations into a sequence encoding GCREC or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:20-38 and fragments thereof under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) *Methods Enzymol.* 152:399-407; Kimmel, A.R. (1987) *Methods Enzymol.* 35 152:507-511.) Hybridization conditions, including annealing and wash conditions, are described in

"Definitions."

Methods for DNA sequencing are well known in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE (US Biochemical, Cleveland OH), Taq polymerase (Applied Biosystems), thermostable T7 polymerase (Amersham Pharmacia Biotech, Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 liquid transfer system (Hamilton, Reno NV), PTC200 thermal cycler (MJ Research, Watertown MA) and ABI CATALYST 800 thermal cycler (Applied Biosystems). Sequencing is then carried out using either the ABI 373 or 377 DNA sequencing system (Applied Biosystems), the MEGABACE 1000 DNA sequencing system (Molecular Dynamics, Sunnyvale CA), or other systems known in the art. The resulting sequences are analyzed using a variety of algorithms which are well known in the art. (See, e.g., Ausubel, F.M. (1997) Short Protocols in Molecular Biology, John Wiley & Sons, New York NY, unit 7.7; Meyers, R.A. (1995) Molecular Biology and Biotechnology, Wiley VCH, New York NY, pp. 856-853.)

The nucleic acid sequences encoding GCREC may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences, such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) *PCR Methods Applic.* 2:318-322.) Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) *Nucleic Acids Res.* 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) *PCR Methods Applic.* 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) *Nucleic Acids Res.* 19:3055-3060). Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries (Clontech, Palo Alto CA) to walk genomic DNA. This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 primer analysis software (National Biosciences, Plymouth MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of



about 68°C to 72°C.

When screening for full length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Applied Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode GCREC may be cloned in recombinant DNA molecules that direct expression of GCREC, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express GCREC.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter GCREC-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

The nucleotides of the present invention may be subjected to DNA shuffling techniques such as MOLECULARBREEDING (Maxygen Inc., Santa Clara CA; described in U.S. Patent Number 5,837,458; Chang, C.-C. et al. (1999) Nat. Biotechnol. 17:793-797; Christians, F.C. et al. (1999) Nat. Biotechnol. 17:259-264; and Crameri, A. et al. (1996) Nat. Biotechnol. 14:315-319) to alter or improve the biological properties of GCREC, such as its biological or enzymatic activity or its ability to bind to other molecules or compounds. DNA shuffling is a process by which a library of gene variants is produced using PCR-mediated recombination of gene fragments. The library is then subjected to selection or screening procedures that identify those gene variants with the desired

properties. These preferred variants may then be pooled and further subjected to recursive rounds of DNA shuffling and selection/screening. Thus, genetic diversity is created through "artificial" breeding and rapid molecular evolution. For example, fragments of a single gene containing random point mutations may be recombined, screened, and then reshuffled until the desired properties are optimized. Alternatively, fragments of a given gene may be recombined with fragments of homologous genes in the same gene family, either from the same or different species, thereby maximizing the genetic diversity of multiple naturally occurring genes in a directed and controllable manner.

In another embodiment, sequences encoding GCREC may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) *Nucleic Acids Symp. Ser.* 7:215-223; and Horn, T. et al. (1980) *Nucleic Acids Symp. Ser.* 7:225-232.)

Alternatively, GCREC itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solution-phase or solid-phase techniques. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman, New York NY, pp. 55-60; and Roberge, J.Y. et al. (1995) *Science* 269:202-204.) Automated synthesis may be achieved using the ABI 431A peptide synthesizer (Applied Biosystems). Additionally, the amino acid sequence of GCREC, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide or a polypeptide having a sequence of a naturally occurring polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) *Methods Enzymol.* 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, supra, pp. 28-53.)

In order to express a biologically active GCREC, the nucleotide sequences encoding GCREC or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding GCREC. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding GCREC. Such signals include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding GCREC and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-

frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) *Results Probl. Cell Differ.* 20:125-162.)

5       Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding GCRC and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview NY, ch. 4, 8, and 16-17; Ausubel, F.M. et al. (1995) Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding GCRC. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast  
15 transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus, CaMV, or tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. (See, e.g., Sambrook, supra; Ausubel, supra; Van Heeke, G. and S.M. Schuster (1989) *J. Biol. Chem.* 264:5503-5509; Engelhard, E.K. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3224-3227; Sandig, V. et al. (1996) *Hum. Gene Ther.* 7:1937-1945; Takamatsu, N. (1987) *EMBO J.* 6:307-311; The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196; Logan, J. and T. Shenk (1984) *Proc. Natl. Acad. Sci. USA* 81:3655-3659; and Harrington, J.J. et al. (1997) *Nat. Genet.* 15:345-355.) Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids,  
25 may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. (See, e.g., Di Nicola, M. et al. (1998) *Cancer Gen. Ther.* 5(6):350-356; Yu, M. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90(13):6340-6344; Buller, R.M. et al. (1985) *Nature* 317(6040):813-815; McGregor, D.P. et al. (1994) *Mol. Immunol.* 31(3):219-226; and Verma, I.M. and N. Somia (1997) *Nature* 389:239-242.) The invention is not limited by the host cell employed.

30       In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding GCRC. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding GCRC can be achieved using a multifunctional E. coli vector such as PBLUESCRIPT (Stratagene, La Jolla CA) or PSORT1 plasmid (Life Technologies). Ligation of sequences encoding GCRC into the vector's multiple  
35 cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of

transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of GCREC are needed, e.g. for the production of antibodies, vectors which direct high level expression of GCREC may be used. For example, vectors containing the strong, inducible SP6 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of GCREC. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH promoters, may be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, 1995, supra; Bitter, G.A. et al. (1987) Methods Enzymol. 153:516-544; and Scorer, C.A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of GCREC. Transcription of sequences encoding GCREC may be driven by viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. (1987) EMBO J. 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., The McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York NY, pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding GCREC may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses GCREC in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. USA 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes. (See, e.g., Harrington, J.J. et al. (1997) Nat. Genet. 15:345-355.)

For long term production of recombinant proteins in mammalian systems, stable expression of GCREC in cell lines is preferred. For example, sequences encoding GCREC can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector.

5 Following the introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

10 Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*<sup>-</sup> and *apr*<sup>-</sup> cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to  
15 methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* and *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77:3567-3570; Colbere-Garapin, F. et al. (1981) J. Mol. Biol. 150:1-14.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc.  
20 Natl. Acad. Sci. USA 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP; Clontech),  $\beta$  glucuronidase and its substrate  $\beta$ -glucuronide, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. (1995) Methods Mol. Biol. 55:121-131.)

25 Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding GCREC is inserted within a marker gene sequence, transformed cells containing sequences encoding GCREC can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding GCREC under the control of a  
30 single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding GCREC and that express GCREC may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR  
35 amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or

chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of GCREC using either specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and  
5 fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on GCREC is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St. Paul MN, Sect. IV; Coligan, J.E. et al. (1997) Current Protocols in Immunology, Greene Pub. Associates and  
10 Wiley-Interscience, New York NY; and Pound, J.D. (1998) Immunochemical Protocols, Humana Press, Totowa NJ.)

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding GCREC  
15 include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding GCREC, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety  
20 of commercially available kits, such as those provided by Amersham Pharmacia Biotech, Promega (Madison WI), and US Biochemical. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding GCREC may be cultured under  
25 conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode GCREC may be designed to contain signal sequences which direct secretion of GCREC through a prokaryotic or eukaryotic cell membrane.

30 In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" or "pro" form of the protein may also be used to specify protein targeting, folding, and/or activity.  
35 Different host cells which have specific cellular machinery and characteristic mechanisms for

post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38) are available from the American Type Culture Collection (ATCC, Manassas VA) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding GCREC may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric GCREC protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of GCREC activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the GCREC encoding sequence and the heterologous protein sequence, so that GCREC may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel (1995, supra, ch. 10). A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled GCREC may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract system (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, for example, <sup>35</sup>S-methionine.

GCREC of the present invention or fragments thereof may be used to screen for compounds that specifically bind to GCREC. At least one and up to a plurality of test compounds may be screened for specific binding to GCREC. Examples of test compounds include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

In one embodiment, the compound thus identified is closely related to the natural ligand of GCREC, e.g., a ligand or fragment thereof, a natural substrate, a structural or functional mimetic, or a natural binding partner. (See, e.g., Coligan, J.E. et al. (1991) Current Protocols in Immunology 1(2): Chapter 5.) Similarly, the compound can be closely related to the natural receptor to which GCREC binds, or to at least a fragment of the receptor, e.g., the ligand binding site. In either case, the

compound can be rationally designed using known techniques. In one embodiment, screening for these compounds involves producing appropriate cells which express GCREC, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing GCREC or cell membrane fractions which contain GCREC are then  
5 contacted with a test compound and binding, stimulation, or inhibition of activity of either GCREC or the compound is analyzed.

An assay may simply test binding of a test compound to the polypeptide, wherein binding is detected by a fluorophore, radioisotope, enzyme conjugate, or other detectable label. For example, the assay may comprise the steps of combining at least one test compound with GCREC, either in  
10 solution or affixed to a solid support, and detecting the binding of GCREC to the compound.

Alternatively, the assay may detect or measure binding of a test compound in the presence of a labeled competitor. Additionally, the assay may be carried out using cell-free preparations, chemical libraries, or natural product mixtures, and the test compound(s) may be free in solution or affixed to a solid support.

15 GCREC of the present invention or fragments thereof may be used to screen for compounds that modulate the activity of GCREC. Such compounds may include agonists, antagonists, or partial or inverse agonists. In one embodiment, an assay is performed under conditions permissive for GCREC activity, wherein GCREC is combined with at least one test compound, and the activity of GCREC in the presence of a test compound is compared with the activity of GCREC in the absence  
20 of the test compound. A change in the activity of GCREC in the presence of the test compound is indicative of a compound that modulates the activity of GCREC. Alternatively, a test compound is combined with an in vitro or cell-free system comprising GCREC under conditions suitable for GCREC activity, and the assay is performed. In either of these assays, a test compound which modulates the activity of GCREC may do so indirectly and need not come in direct contact with the  
25 test compound. At least one and up to a plurality of test compounds may be screened.

In another embodiment, polynucleotides encoding GCREC or their mammalian homologs may be "knocked out" in an animal model system using homologous recombination in embryonic stem (ES) cells. Such techniques are well known in the art and are useful for the generation of animal models of human disease. (See, e.g., U.S. Patent Number 5,175,383 and U.S. Patent Number  
30 5,767,337.) For example, mouse ES cells, such as the mouse 129/SvJ cell line, are derived from the early mouse embryo and grown in culture. The ES cells are transformed with a vector containing the gene of interest disrupted by a marker gene, e.g., the neomycin phosphotransferase gene (neo; Capecchi, M.R. (1989) Science 244:1288-1292). The vector integrates into the corresponding region of the host genome by homologous recombination. Alternatively, homologous recombination takes  
35 place using the Cre-loxP system to knockout a gene of interest in a tissue- or developmental stage-



specific manner (Marth, J.D. (1996) Clin. Invest. 97:1999-2002; Wagner, K.U. et al. (1997) Nucleic Acids Res. 25:4323-4330). Transformed ES cells are identified and microinjected into mouse cell blastocysts such as those from the C57BL/6 mouse strain. The blastocysts are surgically transferred to pseudopregnant dams, and the resulting chimeric progeny are genotyped and bred to produce  
5 heterozygous or homozygous strains. Transgenic animals thus generated may be tested with potential therapeutic or toxic agents.

Polynucleotides encoding GCREC may also be manipulated in vitro in ES cells derived from human blastocysts. Human ES cells have the potential to differentiate into at least eight separate cell lineages including endoderm, mesoderm, and ectodermal cell types. These cell lineages differentiate  
10 into, for example, neural cells, hematopoietic lineages, and cardiomyocytes (Thomson, J.A. et al. (1998) Science 282:1145-1147).

Polynucleotides encoding GCREC can also be used to create "knockin" humanized animals (pigs) or transgenic animals (mice or rats) to model human disease. With knockin technology, a region of a polynucleotide encoding GCREC is injected into animal ES cells, and the injected  
15 sequence integrates into the animal cell genome. Transformed cells are injected into blastulae, and the blastulae are implanted as described above. Transgenic progeny or inbred lines are studied and treated with potential pharmaceutical agents to obtain information on treatment of a human disease. Alternatively, a mammal inbred to overexpress GCREC, e.g., by secreting GCREC in its milk, may also serve as a convenient source of that protein (Janne, J. et al. (1998) Biotechnol. Annu. Rev. 4:55-  
20 74).

## THERAPEUTICS

Chemical and structural similarity, e.g., in the context of sequences and motifs, exists between regions of GCREC and G-protein coupled receptors. In addition, the expression of GCREC is closely associated with brain tissue, fetal brain tissue, colon polyps, diseased colon tissue, colon  
25 tumor tissue, diseased gallbladder tissue, heart tissue, diseased breast tissue, interleukin-5 stimulated eosinophils, tumor tissue, and reproductive tissues. Therefore, GCREC appears to play a role in cell proliferative, neurological, cardiovascular, gastrointestinal, autoimmune/inflammatory, and metabolic disorders, and viral infections. In the treatment of disorders associated with increased GCREC expression or activity, it is desirable to decrease the expression or activity of GCREC. In the  
30 treatment of disorders associated with decreased GCREC expression or activity, it is desirable to increase the expression or activity of GCREC.

Therefore, in one embodiment, GCREC or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of GCREC. Examples of such disorders include, but are not limited to, a cell proliferative  
35 disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed

connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, colon, gall bladder, ganglia, 5 gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms, Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, 10 retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, 15 tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, 20 myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a cardiovascular disorder such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, 25 Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, vascular tumors, complications of thrombolysis, balloon angioplasty, vascular replacement, and coronary artery bypass graft surgery, congestive heart failure, ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease, degenerative valvular heart disease, calcific aortic valve stenosis, congenitally bicuspid aortic valve, mitral annular 30 calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart disease, congenital heart disease, and complications of cardiac transplantation; a gastrointestinal disorder such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma,

- dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the
- 5 liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome, colonic carcinoma, colonic obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha<sub>1</sub>-antitrypsin deficiency,
- 10 Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS),
- 15 Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis,
- 20 erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura,
- 25 ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a metabolic disorder such as diabetes, obesity, and osteoporosis; and an infection by a viral agent classified as adenovirus, arenavirus, bunyavirus, calicivirus, coronavirus, filovirus, hepadnavirus, herpesvirus, flavivirus, orthomyxovirus, parvovirus, papovavirus, paramyxovirus,
- 30 picornavirus, poxvirus, reovirus, retrovirus, rhabdovirus, and togavirus.

In another embodiment, a vector capable of expressing GCREC or a fragment or derivative thereof may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of GCREC including, but not limited to, those described above.

In a further embodiment, a composition comprising a substantially purified GCREC in

conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a disorder associated with decreased expression or activity of GCREC including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of GCREC may be  
5 administered to a subject to treat or prevent a disorder associated with decreased expression or activity of GCREC including, but not limited to, those listed above.

In a further embodiment, an antagonist of GCREC may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of GCREC. Examples of such disorders include, but are not limited to, those cell proliferative, neurological, cardiovascular,  
10 gastrointestinal, autoimmune/inflammatory, and metabolic disorders, and viral infections described above. In one aspect, an antibody which specifically binds GCREC may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissues which express GCREC.

In an additional embodiment, a vector expressing the complement of the polynucleotide  
15 encoding GCREC may be administered to a subject to treat or prevent a disorder associated with increased expression or activity of GCREC including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made  
20 by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of GCREC may be produced using methods which are generally known in the  
25 art. In particular, purified GCREC may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind GCREC. Antibodies to GCREC may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit  
30 dimer formation) are generally preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with GCREC or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral  
35 gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic

polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to GCREC have an amino acid sequence consisting of at least about 5 amino acids, and generally will  
5 consist of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein. Short stretches of GCREC amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

Monoclonal antibodies to GCREC may be prepared using any technique which provides for  
10 the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) Nature 256:495-497; Kozbor, D. et al. (1985) J. Immunol. Methods 81:31-42; Cote, R.J. et al. (1983) Proc. Natl. Acad. Sci. USA 80:2026-2030; and Cole, S.P. et al. (1984) Mol. Cell Biol. 62:109-120.)

15 In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) Proc. Natl. Acad. Sci. USA 81:6851-6855; Neuberger, M.S. et al. (1984) Nature 312:604-608; and Takeda, S. et al. (1985) Nature 314:452-454.) Alternatively, techniques described for the production of single  
20 chain antibodies may be adapted, using methods known in the art, to produce GCREC-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton, D.R. (1991) Proc. Natl. Acad. Sci. USA 88:10134-10137.)

Antibodies may also be produced by inducing in vivo production in the lymphocyte  
25 population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) Proc. Natl. Acad. Sci. USA 86:3833-3837; Winter, G. et al. (1991) Nature 349:293-299.)

Antibody fragments which contain specific binding sites for GCREC may also be generated. For example, such fragments include, but are not limited to, F(ab')<sub>2</sub> fragments produced by pepsin  
30 digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired  
35 specificity. Numerous protocols for competitive binding or immunoradiometric assays using either

polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between GCREC and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering GCREC epitopes is generally used, but a competitive binding assay  
5 may also be employed (Pound, supra).

Various methods such as Scatchard analysis in conjunction with radioimmunoassay techniques may be used to assess the affinity of antibodies for GCREC. Affinity is expressed as an association constant,  $K_a$ , which is defined as the molar concentration of GCREC-antibody complex divided by the molar concentrations of free antigen and free antibody under equilibrium conditions.  
10 The  $K_a$  determined for a preparation of polyclonal antibodies, which are heterogeneous in their affinities for multiple GCREC epitopes, represents the average affinity, or avidity, of the antibodies for GCREC. The  $K_a$  determined for a preparation of monoclonal antibodies, which are monospecific for a particular GCREC epitope, represents a true measure of affinity. High-affinity antibody preparations with  $K_a$  ranging from about  $10^9$  to  $10^{12}$  L/mole are preferred for use in immunoassays in  
15 which the GCREC-antibody complex must withstand rigorous manipulations. Low-affinity antibody preparations with  $K_a$  ranging from about  $10^6$  to  $10^7$  L/mole are preferred for use in immunopurification and similar procedures which ultimately require dissociation of GCREC, preferably in active form, from the antibody (Catty, D. (1988) Antibodies, Volume I: A Practical Approach, IRL Press, Washington DC; Liddell, J.E. and A. Cryer (1991) A Practical Guide to  
20 Monoclonal Antibodies, John Wiley & Sons, New York NY).

The titer and avidity of polyclonal antibody preparations may be further evaluated to determine the quality and suitability of such preparations for certain downstream applications. For example, a polyclonal antibody preparation containing at least 1-2 mg specific antibody/ml, preferably 5-10 mg specific antibody/ml, is generally employed in procedures requiring precipitation  
25 of GCREC-antibody complexes. Procedures for evaluating antibody specificity, titer, and avidity, and guidelines for antibody quality and usage in various applications, are generally available. (See, e.g., Catty, supra, and Coligan et al. supra.)

In another embodiment of the invention, the polynucleotides encoding GCREC, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, modifications  
30 of gene expression can be achieved by designing complementary sequences or antisense molecules (DNA, RNA, PNA, or modified oligonucleotides) to the coding or regulatory regions of the gene encoding GCREC. Such technology is well known in the art, and antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding GCREC. (See, e.g., Agrawal, S., ed. (1996) Antisense Therapeutics, Humana Press Inc.,  
35 Totawa NJ.)

In therapeutic use, any gene delivery system suitable for introduction of the antisense sequences into appropriate target cells can be used. Antisense sequences can be delivered intracellularly in the form of an expression plasmid which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein. (See, e.g.,

5 Slater, J.E. et al. (1998) *J. Allergy Clin. Immunol.* 102(3):469-475; and Scanlon, K.J. et al. (1995) 9(13):1288-1296.) Antisense sequences can also be introduced intracellularly through the use of viral vectors, such as retrovirus and adeno-associated virus vectors. (See, e.g., Miller, A.D. (1990) *Blood* 76:271; Ausubel, *supra*; Uckert, W. and W. Walther (1994) *Pharmacol. Ther.* 63(3):323-347.) Other gene delivery mechanisms include liposome-derived systems, artificial viral envelopes, and other

10 systems known in the art. (See, e.g., Rossi, J.J. (1995) *Br. Med. Bull.* 51(1):217-225; Boado, R.J. et al. (1998) *J. Pharm. Sci.* 87(11):1308-1315; and Morris, M.C. et al. (1997) *Nucleic Acids Res.* 25(14):2730-2736.)

In another embodiment of the invention, polynucleotides encoding GCREC may be used for somatic or germline gene therapy. Gene therapy may be performed to (i) correct a genetic deficiency

15 (e.g., in the cases of severe combined immunodeficiency (SCID)-X1 disease characterized by X-linked inheritance (Cavazzana-Calvo, M. et al. (2000) *Science* 288:669-672), severe combined immunodeficiency syndrome associated with an inherited adenosine deaminase (ADA) deficiency (Blaese, R.M. et al. (1995) *Science* 270:475-480; Bordignon, C. et al. (1995) *Science* 270:470-475), cystic fibrosis (Zabner, J. et al. (1993) *Cell* 75:207-216; Crystal, R.G. et al. (1995) *Hum. Gene*

20 *Therapy* 6:643-666; Crystal, R.G. et al. (1995) *Hum. Gene Therapy* 6:667-703), thalassemias, familial hypercholesterolemia, and hemophilia resulting from Factor VIII or Factor IX deficiencies (Crystal, R.G. (1995) *Science* 270:404-410; Verma, I.M. and N. Somia (1997) *Nature* 389:239-242)), (ii) express a conditionally lethal gene product (e.g., in the case of cancers which result from unregulated cell proliferation), or (iii) express a protein which affords protection against intracellular parasites

25 (e.g., against human retroviruses, such as human immunodeficiency virus (HIV) (Baltimore, D. (1988) *Nature* 335:395-396; Poeschla, E. et al. (1996) *Proc. Natl. Acad. Sci. USA.* 93:11395-11399), hepatitis B or C virus (HBV, HCV); fungal parasites, such as Candida albicans and Paracoccidioides brasiliensis; and protozoan parasites such as Plasmodium falciparum and Trypanosoma cruzi). In the case where a genetic deficiency in GCREC expression or regulation causes disease, the expression of

30 GCREC from an appropriate population of transduced cells may alleviate the clinical manifestations caused by the genetic deficiency.

In a further embodiment of the invention, diseases or disorders caused by deficiencies in GCREC are treated by constructing mammalian expression vectors encoding GCREC and introducing these vectors by mechanical means into GCREC-deficient cells. Mechanical transfer technologies for

35 use with cells in vivo or ex vitro include (i) direct DNA microinjection into individual cells, (ii)

ballistic gold particle delivery, (iii) liposome-mediated transfection, (iv) receptor-mediated gene transfer, and (v) the use of DNA transposons (Morgan, R.A. and W.F. Anderson (1993) *Annu. Rev. Biochem.* 62:191-217; Ivics, Z. (1997) *Cell* 91:501-510; Boulay, J.-L. and H. R  cipon (1998) *Curr. Opin. Biotechnol.* 9:445-450).

- 5           Expression vectors that may be effective for the expression of GCREC include, but are not limited to, the PCDNA 3.1, EPITAG, PRCCMV2, PREP, PVAX, PCR2-TOPOTA vectors (Invitrogen, Carlsbad CA), PCMV-SCRIPT, PCMV-TAG, PEGSH/PERV (Stratagene, La Jolla CA), and PTET-OFF, PTET-ON, PTRE2, PTRE2-LUC, PTK-HYG (Clontech, Palo Alto CA). GCREC may be expressed using (i) a constitutively active promoter, (e.g., from cytomegalovirus (CMV),
- 10   Rous sarcoma virus (RSV), SV40 virus, thymidine kinase (TK), or  $\beta$ -actin genes), (ii) an inducible promoter (e.g., the tetracycline-regulated promoter (Gossen, M. and H. Bujard (1992) *Proc. Natl. Acad. Sci. USA* 89:5547-5551; Gossen, M. et al. (1995) *Science* 268:1766-1769; Rossi, F.M.V. and H.M. Blau (1998) *Curr. Opin. Biotechnol.* 9:451-456), commercially available in the T-REX plasmid (Invitrogen)); the ecdysone-inducible promoter (available in the plasmids PVGRXR and PIND;
- 15   Invitrogen); the FK506/rapamycin inducible promoter; or the RU486/mifepristone inducible promoter (Rossi, F.M.V. and Blau, H.M. *supra*), or (iii) a tissue-specific promoter or the native promoter of the endogenous gene encoding GCREC from a normal individual.

- Commercially available liposome transformation kits (e.g., the PERFECT LIPID TRANSFECTION KIT, available from Invitrogen) allow one with ordinary skill in the art to deliver
- 20   polynucleotides to target cells in culture and require minimal effort to optimize experimental parameters. In the alternative, transformation is performed using the calcium phosphate method (Graham, F.L. and A.J. Eb (1973) *Virology* 52:456-467), or by electroporation (Neumann, E. et al. (1982) *EMBO J.* 1:841-845). The introduction of DNA to primary cells requires modification of these standardized mammalian transfection protocols.

- 25           In another embodiment of the invention, diseases or disorders caused by genetic defects with respect to GCREC expression are treated by constructing a retrovirus vector consisting of (i) the polynucleotide encoding GCREC under the control of an independent promoter or the retrovirus long terminal repeat (LTR) promoter, (ii) appropriate RNA packaging signals, and (iii) a Rev-responsive element (RRE) along with additional retrovirus *cis*-acting RNA sequences and coding sequences
- 30   required for efficient vector propagation. Retrovirus vectors (e.g., PFB and PFBNEO) are commercially available (Stratagene) and are based on published data (Riviere, I. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:6733-6737), incorporated by reference herein. The vector is propagated in an appropriate vector producing cell line (VPCL) that expresses an envelope gene with a tropism for receptors on the target cells or a promiscuous envelope protein such as VSVg (Armentano, D. et al.
- 35   (1987) *J. Virol.* 61:1647-1650; Bender, M.A. et al. (1987) *J. Virol.* 61:1639-1646; Adam, M.A. and



A.D. Miller (1988) *J. Virol.* 62:3802-3806; Dull, T. et al. (1998) *J. Virol.* 72:8463-8471; Zufferey, R. et al. (1998) *J. Virol.* 72:9873-9880). U.S. Patent Number 5,910,434 to Rigg ("Method for obtaining retrovirus packaging cell lines producing high transducing efficiency retroviral supernatant") discloses a method for obtaining retrovirus packaging cell lines and is hereby incorporated by  
5 reference. Propagation of retrovirus vectors, transduction of a population of cells (e.g., CD4<sup>+</sup> T-cells), and the return of transduced cells to a patient are procedures well known to persons skilled in the art of gene therapy and have been well documented (Ranga, U. et al. (1997) *J. Virol.* 71:7020-7029; Bauer, G. et al. (1997) *Blood* 89:2259-2267; Bonyhadi, M.L. (1997) *J. Virol.* 71:4707-4716; Ranga, U. et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:1201-1206; Su, L. (1997) *Blood* 89:2283-  
10 2290).

In the alternative, an adenovirus-based gene therapy delivery system is used to deliver polynucleotides encoding GCREC to cells which have one or more genetic abnormalities with respect to the expression of GCREC. The construction and packaging of adenovirus-based vectors are well known to those with ordinary skill in the art. Replication defective adenovirus vectors have proven to  
15 be versatile for importing genes encoding immunoregulatory proteins into intact islets in the pancreas (Csete, M.E. et al. (1995) *Transplantation* 27:263-268). Potentially useful adenoviral vectors are described in U.S. Patent Number 5,707,618 to Armentano ("Adenovirus vectors for gene therapy"), hereby incorporated by reference. For adenoviral vectors, see also Antinozzi, P.A. et al. (1999) *Annu. Rev. Nutr.* 19:511-544 and Verma, I.M. and N. Somia (1997) *Nature* 18:389:239-242, both  
20 incorporated by reference herein.

In another alternative, a herpes-based, gene therapy delivery system is used to deliver polynucleotides encoding GCREC to target cells which have one or more genetic abnormalities with respect to the expression of GCREC. The use of herpes simplex virus (HSV)-based vectors may be especially valuable for introducing GCREC to cells of the central nervous system, for which HSV has  
25 a tropism. The construction and packaging of herpes-based vectors are well known to those with ordinary skill in the art. A replication-competent herpes simplex virus (HSV) type 1-based vector has been used to deliver a reporter gene to the eyes of primates (Liu, X. et al. (1999) *Exp. Eye Res.* 169:385-395). The construction of a HSV-1 virus vector has also been disclosed in detail in U.S. Patent Number 5,804,413 to DeLuca ("Herpes simplex virus strains for gene transfer"), which is  
30 hereby incorporated by reference. U.S. Patent Number 5,804,413 teaches the use of recombinant HSV d92 which consists of a genome containing at least one exogenous gene to be transferred to a cell under the control of the appropriate promoter for purposes including human gene therapy. Also taught by this patent are the construction and use of recombinant HSV strains deleted for ICP4, ICP27 and ICP22. For HSV vectors, see also Goins, W.F. et al. (1999) *J. Virol.* 73:519-532 and Xu, H. et al.  
35 (1994) *Dev. Biol.* 163:152-161, hereby incorporated by reference. The manipulation of cloned

herpesvirus sequences, the generation of recombinant virus following the transfection of multiple plasmids containing different segments of the large herpesvirus genomes, the growth and propagation of herpesvirus, and the infection of cells with herpesvirus are techniques well known to those of ordinary skill in the art.

5 In another alternative, an alphavirus (positive, single-stranded RNA virus) vector is used to deliver polynucleotides encoding GCREC to target cells. The biology of the prototypic alphavirus, Semliki Forest Virus (SFV), has been studied extensively and gene transfer vectors have been based on the SFV genome (Garoff, H. and K.-J. Li (1998) *Curr. Opin. Biotechnol.* 9:464-469). During alphavirus RNA replication, a subgenomic RNA is generated that normally encodes the viral capsid  
10 proteins. This subgenomic RNA replicates to higher levels than the full length genomic RNA, resulting in the overproduction of capsid proteins relative to the viral proteins with enzymatic activity (e.g., protease and polymerase). Similarly, inserting the coding sequence for GCREC into the alphavirus genome in place of the capsid-coding region results in the production of a large number of GCREC-coding RNAs and the synthesis of high levels of GCREC in vector transduced cells. While  
15 alphavirus infection is typically associated with cell lysis within a few days, the ability to establish a persistent infection in hamster normal kidney cells (BHK-21) with a variant of Sindbis virus (SIN) indicates that the lytic replication of alphaviruses can be altered to suit the needs of the gene therapy application (Dryga, S.A. et al. (1997) *Virology* 228:74-83). The wide host range of alphaviruses will allow the introduction of GCREC into a variety of cell types. The specific transduction of a subset of  
20 cells in a population may require the sorting of cells prior to transduction. The methods of manipulating infectious cDNA clones of alphaviruses, performing alphavirus cDNA and RNA transfections, and performing alphavirus infections, are well known to those with ordinary skill in the art.

Oligonucleotides derived from the transcription initiation site, e.g., between about positions  
25 -10 and +10 from the start site, may also be employed to inhibit gene expression. Similarly, inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E.  
30 and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing, Mt. Kisco NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme  
35 molecule to complementary target RNA, followed by endonucleolytic cleavage. For example,

engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding GCRC.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA,  
5 GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

10 Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding GCRC. Such DNA sequences may be incorporated into a wide variety of  
15 vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3'  
20 ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous  
25 endonucleases.

An additional embodiment of the invention encompasses a method for screening for a compound which is effective in altering expression of a polynucleotide encoding GCRC. Compounds which may be effective in altering expression of a specific polynucleotide may include, but are not limited to, oligonucleotides, antisense oligonucleotides, triple helix-forming  
30 oligonucleotides, transcription factors and other polypeptide transcriptional regulators, and non-macromolecular chemical entities which are capable of interacting with specific polynucleotide sequences. Effective compounds may alter polynucleotide expression by acting as either inhibitors or promoters of polynucleotide expression. Thus, in the treatment of disorders associated with increased GCRC expression or activity, a compound which specifically inhibits expression of the  
35 polynucleotide encoding GCRC may be therapeutically useful, and in the treatment of disorders

associated with decreased GCREC expression or activity, a compound which specifically promotes expression of the polynucleotide encoding GCREC may be therapeutically useful.

At least one, and up to a plurality, of test compounds may be screened for effectiveness in altering expression of a specific polynucleotide. A test compound may be obtained by any method  
5 commonly known in the art, including chemical modification of a compound known to be effective in altering polynucleotide expression; selection from an existing, commercially-available or proprietary library of naturally-occurring or non-natural chemical compounds; rational design of a compound based on chemical and/or structural properties of the target polynucleotide; and selection from a library of chemical compounds created combinatorially or randomly. A sample comprising a  
10 polynucleotide encoding GCREC is exposed to at least one test compound thus obtained. The sample may comprise, for example, an intact or permeabilized cell, or an in vitro cell-free or reconstituted biochemical system. Alterations in the expression of a polynucleotide encoding GCREC are assayed by any method commonly known in the art. Typically, the expression of a specific nucleotide is detected by hybridization with a probe having a nucleotide sequence complementary to the sequence  
15 of the polynucleotide encoding GCREC. The amount of hybridization may be quantified, thus forming the basis for a comparison of the expression of the polynucleotide both with and without exposure to one or more test compounds. Detection of a change in the expression of a polynucleotide exposed to a test compound indicates that the test compound is effective in altering the expression of the polynucleotide. A screen for a compound effective in altering expression of a specific  
20 polynucleotide can be carried out, for example, using a Schizosaccharomyces pombe gene expression system (Atkins, D. et al. (1999) U.S. Patent No. 5,932,435; Arndt, G.M. et al. (2000) Nucleic Acids Res. 28:E15) or a human cell line such as HeLa cell (Clarke, M.L. et al. (2000) Biochem. Biophys. Res. Commun. 268:8-13). A particular embodiment of the present invention involves screening a combinatorial library of oligonucleotides (such as deoxyribonucleotides, ribonucleotides, peptide  
25 nucleic acids, and modified oligonucleotides) for antisense activity against a specific polynucleotide sequence (Bruce, T.W. et al. (1997) U.S. Patent No. 5,686,242; Bruce, T.W. et al. (2000) U.S. Patent No. 6,022,691).

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells  
30 taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) Nat. Biotechnol. 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of

such therapy, including, for example, mammals such as humans, dogs, cats, cows, horses, rabbits, and monkeys.

An additional embodiment of the invention relates to the administration of a composition which generally comprises an active ingredient formulated with a pharmaceutically acceptable  
5 excipient. Excipients may include, for example, sugars, starches, celluloses, gums, and proteins. Various formulations are commonly known and are thoroughly discussed in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing, Easton PA). Such compositions may consist of GCREC, antibodies to GCREC, and mimetics, agonists, antagonists, or inhibitors of GCREC.

10 The compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, pulmonary, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

Compositions for pulmonary administration may be prepared in liquid or dry powder form.

15 These compositions are generally aerosolized immediately prior to inhalation by the patient. In the case of small molecules (e.g. traditional low molecular weight organic drugs), aerosol delivery of fast-acting formulations is well-known in the art. In the case of macromolecules (e.g. larger peptides and proteins), recent developments in the field of pulmonary delivery via the alveolar region of the lung have enabled the practical delivery of drugs such as insulin to blood circulation (see, e.g., Patton,  
20 J.S. et al., U.S. Patent No. 5,997,848). Pulmonary delivery has the advantage of administration without needle injection, and obviates the need for potentially toxic penetration enhancers.

Compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

25 Specialized forms of compositions may be prepared for direct intracellular delivery of macromolecules comprising GCREC or fragments thereof. For example, liposome preparations containing a cell-impermeable macromolecule may promote cell fusion and intracellular delivery of the macromolecule. Alternatively, GCREC or a fragment thereof may be joined to a short cationic N-terminal portion from the HIV Tat-1 protein. Fusion proteins thus generated have been found to  
30 transduce into the cells of all tissues, including the brain, in a mouse model system (Schwarze, S.R. et al. (1999) Science 285:1569-1572).

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models such as mice, rats, rabbits, dogs, monkeys, or pigs. An animal model may also be used to determine the appropriate concentration  
35 range and route of administration. Such information can then be used to determine useful doses and

routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example GCREC or fragments thereof, antibodies of GCREC, and agonists, antagonists or inhibitors of GCREC, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be  
5 determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the  $ED_{50}$  (the dose therapeutically effective in 50% of the population) or  $LD_{50}$  (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the  $LD_{50}/ED_{50}$  ratio. Compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are  
10 used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the  $ED_{50}$  with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the  
15 subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting compositions may be administered every 3 to 4 days, every week,  
20 or biweekly depending on the half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from about 0.1  $\mu\text{g}$  to 100,000  $\mu\text{g}$ , up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their  
25 inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions, locations, etc.

## DIAGNOSTICS

In another embodiment, antibodies which specifically bind GCREC may be used for the diagnosis of disorders characterized by expression of GCREC, or in assays to monitor patients being  
30 treated with GCREC or agonists, antagonists, or inhibitors of GCREC. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for GCREC include methods which utilize the antibody and a label to detect GCREC in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter  
35 molecule. A wide variety of reporter molecules, several of which are described above, are known in

the art and may be used.

A variety of protocols for measuring GCREC, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of GCREC expression. Normal or standard values for GCREC expression are established by combining body fluids or cell  
5 extracts taken from normal mammalian subjects, for example, human subjects, with antibodies to GCREC under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, such as photometric means. Quantities of GCREC expressed in subject, control, and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for  
10 diagnosing disease.

In another embodiment of the invention, the polynucleotides encoding GCREC may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantify gene expression in biopsied tissues in which expression of GCREC may be correlated  
15 with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of GCREC, and to monitor regulation of GCREC levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding GCREC or closely related molecules may be used to identify nucleic acid sequences which encode GCREC. The specificity of the probe, whether it is  
20 made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification will determine whether the probe identifies only naturally occurring sequences encoding GCREC, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and may have at least 50%  
25 sequence identity to any of the GCREC encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:20-38 or from genomic sequences including promoters, enhancers, and introns of the GCREC gene.

Means for producing specific hybridization probes for DNAs encoding GCREC include the cloning of polynucleotide sequences encoding GCREC or GCREC derivatives into vectors for the  
30 production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as <sup>32</sup>P or <sup>35</sup>S, or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

35 Polynucleotide sequences encoding GCREC may be used for the diagnosis of disorders

associated with expression of GCREC. Examples of such disorders include, but are not limited to, a cell proliferative disorder such as actinic keratosis, arteriosclerosis, atherosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, psoriasis, primary thrombocythemia, and cancers including

5 adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, colon, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; a neurological disorder such as epilepsy, ischemic cerebrovascular disease, stroke, cerebral neoplasms,

10 Alzheimer's disease, Pick's disease, Huntington's disease, dementia, Parkinson's disease and other extrapyramidal disorders, amyotrophic lateral sclerosis and other motor neuron disorders, progressive neural muscular atrophy, retinitis pigmentosa, hereditary ataxias, multiple sclerosis and other demyelinating diseases, bacterial and viral meningitis, brain abscess, subdural empyema, epidural abscess, suppurative intracranial thrombophlebitis, myelitis and radiculitis, viral central nervous

15 system disease, prion diseases including kuru, Creutzfeldt-Jakob disease, and Gerstmann-Straussler-Scheinker syndrome, fatal familial insomnia, nutritional and metabolic diseases of the nervous system, neurofibromatosis, tuberous sclerosis, cerebelloretinal hemangioblastomatosis, encephalotrigeminal syndrome, mental retardation and other developmental disorders of the central nervous system, cerebral palsy, neuroskeletal disorders, autonomic nervous system disorders, cranial

20 nerve disorders, spinal cord diseases, muscular dystrophy and other neuromuscular disorders, peripheral nervous system disorders, dermatomyositis and polymyositis, inherited, metabolic, endocrine, and toxic myopathies, myasthenia gravis, periodic paralysis, mental disorders including mood, anxiety, and schizophrenic disorders, seasonal affective disorder (SAD), akathisia, amnesia, catatonia, diabetic neuropathy, tardive dyskinesia, dystonias, paranoid psychoses, postherpetic

25 neuralgia, Tourette's disorder, progressive supranuclear palsy, corticobasal degeneration, and familial frontotemporal dementia; a cardiovascular disorder such as arteriovenous fistula, atherosclerosis, hypertension, vasculitis, Raynaud's disease, aneurysms, arterial dissections, varicose veins, thrombophlebitis and phlebothrombosis, vascular tumors, complications of thrombolysis, balloon angioplasty, vascular replacement, and coronary artery bypass graft surgery, congestive heart failure,

30 ischemic heart disease, angina pectoris, myocardial infarction, hypertensive heart disease, degenerative valvular heart disease, calcific aortic valve stenosis, congenitally bicuspid aortic valve, mitral annular calcification, mitral valve prolapse, rheumatic fever and rheumatic heart disease, infective endocarditis, nonbacterial thrombotic endocarditis, endocarditis of systemic lupus erythematosus, carcinoid heart disease, cardiomyopathy, myocarditis, pericarditis, neoplastic heart



disease, congenital heart disease, and complications of cardiac transplantation; a gastrointestinal disorder such as dysphagia, peptic esophagitis, esophageal spasm, esophageal stricture, esophageal carcinoma, dyspepsia, indigestion, gastritis, gastric carcinoma, anorexia, nausea, emesis, gastroparesis, antral or pyloric edema, abdominal angina, pyrosis, gastroenteritis, intestinal

5 obstruction, infections of the intestinal tract, peptic ulcer, cholelithiasis, cholecystitis, cholestasis, pancreatitis, pancreatic carcinoma, biliary tract disease, hepatitis, hyperbilirubinemia, cirrhosis, passive congestion of the liver, hepatoma, infectious colitis, ulcerative colitis, ulcerative proctitis, Crohn's disease, Whipple's disease, Mallory-Weiss syndrome, colonic carcinoma, colonic

10 obstruction, irritable bowel syndrome, short bowel syndrome, diarrhea, constipation, gastrointestinal hemorrhage, acquired immunodeficiency syndrome (AIDS) enteropathy, jaundice, hepatic encephalopathy, hepatorenal syndrome, hepatic steatosis, hemochromatosis, Wilson's disease, alpha<sub>1</sub>-antitrypsin deficiency, Reye's syndrome, primary sclerosing cholangitis, liver infarction, portal vein obstruction and thrombosis, centrilobular necrosis, peliosis hepatis, hepatic vein thrombosis, veno-occlusive disease, preeclampsia, eclampsia, acute fatty liver of pregnancy, intrahepatic cholestasis of

15 pregnancy, and hepatic tumors including nodular hyperplasias, adenomas, and carcinomas; an autoimmune/inflammatory disorder such as acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED), bronchitis,

20 cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis,

25 polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; a metabolic disorder such as diabetes, obesity, and osteoporosis; and an infection by a viral

30 agent classified as adenovirus, arenavirus, bunyavirus, calicivirus, coronavirus, filovirus, hepadnavirus, herpesvirus, flavivirus, orthomyxovirus, parvovirus, papovavirus, paramyxovirus, picornavirus, poxvirus, reovirus, retrovirus, rhabdovirus, and togavirus. The polynucleotide sequences encoding GCREC may be used in Southern or northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and multiformat ELISA-like

assays; and in microarrays utilizing fluids or tissues from patients to detect altered GCREC expression. Such qualitative or quantitative methods are well known in the art.

In a particular aspect, the nucleotide sequences encoding GCREC may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding GCREC may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantified and compared with a standard value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding GCREC in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of GCREC, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding GCREC, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of an abnormal amount of transcript (either under- or overexpressed) in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding GCREC may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced *in vitro*. Oligomers will preferably contain a fragment of a polynucleotide

encoding GCREC, or a fragment of a polynucleotide complementary to the polynucleotide encoding GCREC, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantification of closely related DNA or RNA sequences.

5           In a particular aspect, oligonucleotide primers derived from the polynucleotide sequences encoding GCREC may be used to detect single nucleotide polymorphisms (SNPs). SNPs are substitutions, insertions and deletions that are a frequent cause of inherited or acquired genetic disease in humans. Methods of SNP detection include, but are not limited to, single-stranded conformation polymorphism (SSCP) and fluorescent SSCP (fSSCP) methods. In SSCP,  
10   oligonucleotide primers derived from the polynucleotide sequences encoding GCREC are used to amplify DNA using the polymerase chain reaction (PCR). The DNA may be derived, for example, from diseased or normal tissue, biopsy samples, bodily fluids, and the like. SNPs in the DNA cause differences in the secondary and tertiary structures of PCR products in single-stranded form, and these differences are detectable using gel electrophoresis in non-denaturing gels. In fSSCP, the  
15   oligonucleotide primers are fluorescently labeled, which allows detection of the amplimers in high-throughput equipment such as DNA sequencing machines. Additionally, sequence database analysis methods, termed *in silico* SNP (isSNP), are capable of identifying polymorphisms by comparing the sequence of individual overlapping DNA fragments which assemble into a common consensus sequence. These computer-based methods filter out sequence variations due to laboratory preparation  
20   of DNA and sequencing errors using statistical models and automated analyses of DNA sequence chromatograms. In the alternative, SNPs may be detected and characterized by mass spectrometry using, for example, the high throughput MASSARRAY system (Sequenom, Inc., San Diego CA).

          Methods which may also be used to quantify the expression of GCREC include radiolabeling or biotinylating nucleotides, coamplification of a control nucleic acid, and interpolating results from  
25   standard curves. (See, e.g., Melby, P.C. et al. (1993) *J. Immunol. Methods* 159:235-244; Duplaa, C. et al. (1993) *Anal. Biochem.* 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in a high-throughput format where the oligomer or polynucleotide of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

30           In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as elements on a microarray. The microarray can be used in transcript imaging techniques which monitor the relative expression levels of large numbers of genes simultaneously as described below. The microarray may also be used to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene  
35   function, to understand the genetic basis of a disorder, to diagnose a disorder, to monitor

progression/regression of disease as a function of gene expression, and to develop and monitor the activities of therapeutic agents in the treatment of disease. In particular, this information may be used to develop a pharmacogenomic profile of a patient in order to select the most appropriate and effective treatment regimen for that patient. For example, therapeutic agents which are highly effective and display the fewest side effects may be selected for a patient based on his/her pharmacogenomic profile.

In another embodiment, GCREC, fragments of GCREC, or antibodies specific for GCREC may be used as elements on a microarray. The microarray may be used to monitor or measure protein-protein interactions, drug-target interactions, and gene expression profiles, as described above.

A particular embodiment relates to the use of the polynucleotides of the present invention to generate a transcript image of a tissue or cell type. A transcript image represents the global pattern of gene expression by a particular tissue or cell type. Global gene expression patterns are analyzed by quantifying the number of expressed genes and their relative abundance under given conditions and at a given time. (See Seilhamer et al., "Comparative Gene Transcript Analysis," U.S. Patent Number 5,840,484, expressly incorporated by reference herein.) Thus a transcript image may be generated by hybridizing the polynucleotides of the present invention or their complements to the totality of transcripts or reverse transcripts of a particular tissue or cell type. In one embodiment, the hybridization takes place in high-throughput format, wherein the polynucleotides of the present invention or their complements comprise a subset of a plurality of elements on a microarray. The resultant transcript image would provide a profile of gene activity.

Transcript images may be generated using transcripts isolated from tissues, cell lines, biopsies, or other biological samples. The transcript image may thus reflect gene expression *in vivo*, as in the case of a tissue or biopsy sample, or *in vitro*, as in the case of a cell line.

Transcript images which profile the expression of the polynucleotides of the present invention may also be used in conjunction with *in vitro* model systems and preclinical evaluation of pharmaceuticals, as well as toxicological testing of industrial and naturally-occurring environmental compounds. All compounds induce characteristic gene expression patterns, frequently termed molecular fingerprints or toxicant signatures, which are indicative of mechanisms of action and toxicity (Nuwaysir, E.F. et al. (1999) Mol. Carcinog. 24:153-159; Steiner, S. and N.L. Anderson (2000) Toxicol. Lett. 112-113:467-471, expressly incorporated by reference herein). If a test compound has a signature similar to that of a compound with known toxicity, it is likely to share those toxic properties. These fingerprints or signatures are most useful and refined when they contain expression information from a large number of genes and gene families. Ideally, a genome-wide measurement of expression provides the highest quality signature. Even genes whose expression is

not altered by any tested compounds are important as well, as the levels of expression of these genes are used to normalize the rest of the expression data. The normalization procedure is useful for comparison of expression data after treatment with different compounds. While the assignment of gene function to elements of a toxicant signature aids in interpretation of toxicity mechanisms, knowledge of gene function is not necessary for the statistical matching of signatures which leads to prediction of toxicity. (See, for example, Press Release 00-02 from the National Institute of Environmental Health Sciences, released February 29, 2000, available at <http://www.niehs.nih.gov/oc/news/toxchip.htm>.) Therefore, it is important and desirable in toxicological screening using toxicant signatures to include all expressed gene sequences.

In one embodiment, the toxicity of a test compound is assessed by treating a biological sample containing nucleic acids with the test compound. Nucleic acids that are expressed in the treated biological sample are hybridized with one or more probes specific to the polynucleotides of the present invention, so that transcript levels corresponding to the polynucleotides of the present invention may be quantified. The transcript levels in the treated biological sample are compared with levels in an untreated biological sample. Differences in the transcript levels between the two samples are indicative of a toxic response caused by the test compound in the treated sample.

Another particular embodiment relates to the use of the polypeptide sequences of the present invention to analyze the proteome of a tissue or cell type. The term proteome refers to the global pattern of protein expression in a particular tissue or cell type. Each protein component of a proteome can be subjected individually to further analysis. Proteome expression patterns, or profiles, are analyzed by quantifying the number of expressed proteins and their relative abundance under given conditions and at a given time. A profile of a cell's proteome may thus be generated by separating and analyzing the polypeptides of a particular tissue or cell type. In one embodiment, the separation is achieved using two-dimensional gel electrophoresis, in which proteins from a sample are separated by isoelectric focusing in the first dimension, and then according to molecular weight by sodium dodecyl sulfate slab gel electrophoresis in the second dimension (Steiner and Anderson, *supra*). The proteins are visualized in the gel as discrete and uniquely positioned spots, typically by staining the gel with an agent such as Coomassie Blue or silver or fluorescent stains. The optical density of each protein spot is generally proportional to the level of the protein in the sample. The optical densities of equivalently positioned protein spots from different samples, for example, from biological samples either treated or untreated with a test compound or therapeutic agent, are compared to identify any changes in protein spot density related to the treatment. The proteins in the spots are partially sequenced using, for example, standard methods employing chemical or enzymatic cleavage followed by mass spectrometry. The identity of the protein in a spot may be determined by comparing its partial sequence, preferably of at least 5 contiguous amino acid residues, to the

polypeptide sequences of the present invention. In some cases, further sequence data may be obtained for definitive protein identification.

A proteomic profile may also be generated using antibodies specific for GCREC to quantify the levels of GCREC expression. In one embodiment, the antibodies are used as elements on a  
5 microarray, and protein expression levels are quantified by exposing the microarray to the sample and detecting the levels of protein bound to each array element (Lueking, A. et al. (1999) *Anal. Biochem.* 270:103-111; Mendoz, L.G. et al. (1999) *Biotechniques* 27:778-788). Detection may be performed by a variety of methods known in the art, for example, by reacting the proteins in the sample with a thiol- or amino-reactive fluorescent compound and detecting the amount of fluorescence bound at  
10 each array element.

Toxicant signatures at the proteome level are also useful for toxicological screening, and should be analyzed in parallel with toxicant signatures at the transcript level. There is a poor correlation between transcript and protein abundances for some proteins in some tissues (Anderson, N.L. and J. Seilhamer (1997) *Electrophoresis* 18:533-537), so proteome toxicant signatures may be  
15 useful in the analysis of compounds which do not significantly affect the transcript image, but which alter the proteomic profile. In addition, the analysis of transcripts in body fluids is difficult, due to rapid degradation of mRNA, so proteomic profiling may be more reliable and informative in such cases.

In another embodiment, the toxicity of a test compound is assessed by treating a biological  
20 sample containing proteins with the test compound. Proteins that are expressed in the treated biological sample are separated so that the amount of each protein can be quantified. The amount of each protein is compared to the amount of the corresponding protein in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample. Individual proteins are identified by sequencing  
25 the amino acid residues of the individual proteins and comparing these partial sequences to the polypeptides of the present invention.

In another embodiment, the toxicity of a test compound is assessed by treating a biological sample containing proteins with the test compound. Proteins from the biological sample are incubated with antibodies specific to the polypeptides of the present invention. The amount of  
30 protein recognized by the antibodies is quantified. The amount of protein in the treated biological sample is compared with the amount in an untreated biological sample. A difference in the amount of protein between the two samples is indicative of a toxic response to the test compound in the treated sample.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g.,  
35 Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) *Proc. Natl. Acad. Sci.*

USA 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. USA 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.) Various types of microarrays are well known and thoroughly described in DNA Microarrays: A Practical Approach, M. Schena, ed. 5 (1999) Oxford University Press, London, hereby expressly incorporated by reference.

In another embodiment of the invention, nucleic acid sequences encoding GCREC may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. Either coding or noncoding sequences may be used, and in some instances, noncoding sequences may be preferable over coding sequences. For example, conservation of a coding sequence among 10 members of a multi-gene family may potentially cause undesired cross hybridization during chromosomal mapping. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Harrington, J.J. 15 et al. (1997) Nat. Genet. 15:345-355; Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.) Once mapped, the nucleic acid sequences of the invention may be used to develop genetic linkage maps, for example, which correlate the inheritance of a disease state with the inheritance of a particular chromosome region or restriction fragment length polymorphism (RFLP). (See, for example, Lander, E.S. and D. Botstein (1986) Proc. Natl. Acad. Sci. USA 83:7353- 20 7357.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, supra, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) World Wide Web site. Correlation between the location of the gene encoding GCREC on a 25 physical map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder and thus may further positional cloning efforts.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, 30 may reveal associated markers even if the exact chromosomal locus is not known. This information is valuable to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the gene or genes responsible for a disease or syndrome have been crudely localized by genetic linkage to a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further 35 investigation. (See, e.g., Gatti, R.A. et al. (1988) Nature 336:577-580.) The nucleotide sequence of

the instant invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, GCREC, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between GCREC and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate. The test compounds are reacted with GCREC, or fragments thereof, and washed. Bound GCREC is then detected by methods well known in the art. Purified GCREC can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding GCREC specifically compete with a test compound for binding GCREC. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with GCREC.

In additional embodiments, the nucleotide sequences which encode GCREC may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following preferred specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever.

The disclosures of all patents, applications, and publications mentioned above and below, in particular U.S. Ser. No. 60/221,478, U.S. Ser. No. 60/223,268, U.S. Ser. No. 60/231,121, U.S. Ser. No. 60/232,691, U.S. Ser. No. 60/235,146, U.S. Ser. No. 60/227,054, and U.S. Ser. No. 60/232,243, are hereby expressly incorporated by reference.

## EXAMPLES

### I. Construction of cDNA Libraries

Incyte cDNAs were derived from cDNA libraries described in the LIFESEQ GOLD database



(Incyte Genomics, Palo Alto CA) and shown in Table 4, column 5. Some tissues were homogenized and lysed in guanidinium isothiocyanate, while others were homogenized and lysed in phenol or in a suitable mixture of denaturants, such as TRIZOL (Life Technologies), a monophasic solution of phenol and guanidine isothiocyanate. The resulting lysates were centrifuged over CsCl cushions or  
5 extracted with chloroform. RNA was precipitated from the lysates with either isopropanol or sodium acetate and ethanol, or by other routine methods.

Phenol extraction and precipitation of RNA were repeated as necessary to increase RNA purity. In some cases, RNA was treated with DNase. For most libraries, poly(A)+ RNA was isolated using oligo d(T)-coupled paramagnetic particles (Promega), OLIGOTEX latex particles (QIAGEN,  
10 Chatsworth CA), or an OLIGOTEX mRNA purification kit (QIAGEN). Alternatively, RNA was isolated directly from tissue lysates using other RNA isolation kits, e.g., the POLY(A)PURE mRNA purification kit (Ambion, Austin TX).

In some cases, Stratagene was provided with RNA and constructed the corresponding cDNA libraries. Otherwise, cDNA was synthesized and cDNA libraries were constructed with the UNIZAP  
15 vector system (Stratagene) or SUPERScript plasmid system (Life Technologies), using the recommended procedures or similar methods known in the art. (See, e.g., Ausubel, 1997, supra, units 5.1-6.6.) Reverse transcription was initiated using oligo d(T) or random primers. Synthetic oligonucleotide adapters were ligated to double stranded cDNA, and the cDNA was digested with the appropriate restriction enzyme or enzymes. For most libraries, the cDNA was size-selected (300-  
20 1000 bp) using SEPHACRYL S1000, SEPHAROSE CL2B, or SEPHAROSE CL4B column chromatography (Amersham Pharmacia Biotech) or preparative agarose gel electrophoresis. cDNAs were ligated into compatible restriction enzyme sites of the polylinker of a suitable plasmid, e.g., PBLUESCRIPT plasmid (Stratagene), PSPORT1 plasmid (Life Technologies), PCDNA2.1 plasmid (Invitrogen, Carlsbad CA), PBK-CMV plasmid (Stratagene), or pINCY (Incyte Genomics, Palo Alto  
25 CA), or derivatives thereof. Recombinant plasmids were transformed into competent *E. coli* cells including XL1-Blue, XL1-BlueMRF, or SOLR from Stratagene or DH5 $\alpha$ , DH10B, or ElectroMAX DH10B from Life Technologies.

## II. Isolation of cDNA Clones

Plasmids obtained as described in Example I were recovered from host cells by in vivo  
30 excision using the UNIZAP vector system (Stratagene) or by cell lysis. Plasmids were purified using at least one of the following: a Magic or WIZARD Minipreps DNA purification system (Promega); an AGTC Miniprep purification kit (Edge Biosystems, Gaithersburg MD); and QIAWELL 8 Plasmid, QIAWELL 8 Plus Plasmid, QIAWELL 8 Ultra Plasmid purification systems or the R.E.A.L. PREP 96 plasmid purification kit from QIAGEN. Following precipitation, plasmids were resuspended in 0.1  
35 ml of distilled water and stored, with or without lyophilization, at 4°C.

Alternatively, plasmid DNA was amplified from host cell lysates using direct link PCR in a high-throughput format (Rao, V.B. (1994) Anal. Biochem. 216:1-14). Host cell lysis and thermal cycling steps were carried out in a single reaction mixture. Samples were processed and stored in 384-well plates, and the concentration of amplified plasmid DNA was quantified fluorometrically using PICOGREEN dye (Molecular Probes, Eugene OR) and a FLUOROSKAN II fluorescence scanner (Labsystems Oy, Helsinki, Finland).

### III. Sequencing and Analysis

Incyte cDNA recovered in plasmids as described in Example II were sequenced as follows. Sequencing reactions were processed using standard methods or high-throughput instrumentation such as the ABI CATALYST 800 (Applied Biosystems) thermal cyclor or the PTC-200 thermal cyclor (MJ Research) in conjunction with the HYDRA microdispenser (Robbins Scientific) or the MICROLAB 2200 (Hamilton) liquid transfer system. cDNA sequencing reactions were prepared using reagents provided by Amersham Pharmacia Biotech or supplied in ABI sequencing kits such as the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems). Electrophoretic separation of cDNA sequencing reactions and detection of labeled polynucleotides were carried out using the MEGABACE 1000 DNA sequencing system (Molecular Dynamics); the ABI PRISM 373 or 377 sequencing system (Applied Biosystems) in conjunction with standard ABI protocols and base calling software; or other sequence analysis systems known in the art. Reading frames within the cDNA sequences were identified using standard methods (reviewed in Ausubel, 1997, *supra*, unit 7.7). Some of the cDNA sequences were selected for extension using the techniques disclosed in Example VIII.

The polynucleotide sequences derived from Incyte cDNAs were validated by removing vector, linker, and poly(A) sequences and by masking ambiguous bases, using algorithms and programs based on BLAST, dynamic programming, and dinucleotide nearest neighbor analysis. The Incyte cDNA sequences or translations thereof were then queried against a selection of public databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases, and BLOCKS, PRINTS, DOMO, PRODOM, and hidden Markov model (HMM)-based protein family databases such as PFAM. (HMM is a probabilistic approach which analyzes consensus primary structures of gene families. See, for example, Eddy, S.R. (1996) Curr. Opin. Struct. Biol. 6:361-365.) The queries were performed using programs based on BLAST, FASTA, BLIMPS, and HMMER. The Incyte cDNA sequences were assembled to produce full length polynucleotide sequences. Alternatively, GenBank cDNAs, GenBank ESTs, stitched sequences, stretched sequences, or Genscan-predicted coding sequences (see Examples IV and V) were used to extend Incyte cDNA assemblages to full length. Assembly was performed using programs based on Phred, Phrap, and Consed, and cDNA assemblages were screened for open reading frames using programs based on

GeneMark, BLAST, and FASTA. The full length polynucleotide sequences were translated to derive the corresponding full length polypeptide sequences. Alternatively, a polypeptide of the invention may begin at any of the methionine residues of the full length translated polypeptide. Full length polypeptide sequences were subsequently analyzed by querying against databases such as the  
5 GenBank protein databases (genpept), SwissProt, BLOCKS, PRINTS, DOMO, PRODOM, Prosite, and hidden Markov model (HMM)-based protein family databases such as PFAM. Full length polynucleotide sequences are also analyzed using MACDNASIS PRO software (Hitachi Software Engineering, South San Francisco CA) and LASERGENE software (DNASTAR). Polynucleotide and polypeptide sequence alignments are generated using default parameters specified by the  
10 CLUSTAL algorithm as incorporated into the MEGALIGN multisequence alignment program (DNASTAR), which also calculates the percent identity between aligned sequences.

Table 7 summarizes the tools, programs, and algorithms used for the analysis and assembly of Incyte cDNA and full length sequences and provides applicable descriptions, references, and threshold parameters. The first column of Table 7 shows the tools, programs, and algorithms used,  
15 the second column provides brief descriptions thereof, the third column presents appropriate references, all of which are incorporated by reference herein in their entirety, and the fourth column presents, where applicable, the scores, probability values, and other parameters used to evaluate the strength of a match between two sequences (the higher the score or the lower the probability value, the greater the identity between two sequences).

20 The programs described above for the assembly and analysis of full length polynucleotide and polypeptide sequences were also used to identify polynucleotide sequence fragments from SEQ ID NO:20-38. Fragments from about 20 to about 4000 nucleotides which are useful in hybridization and amplification technologies are described in Table 4, column 4.

#### IV. Identification and Editing of Coding Sequences from Genomic DNA

25 Putative G-protein coupled receptors were initially identified by running the Genscan gene identification program against public genomic sequence databases (e.g., gbpri and gbhtg). Genscan is a general-purpose gene identification program which analyzes genomic DNA sequences from a variety of organisms (See Burge, C. and S. Karlin (1997) *J. Mol. Biol.* 268:78-94, and Burge, C. and S. Karlin (1998) *Curr. Opin. Struct. Biol.* 8:346-354). The program concatenates predicted exons to  
30 form an assembled cDNA sequence extending from a methionine to a stop codon. The output of Genscan is a FASTA database of polynucleotide and polypeptide sequences. The maximum range of sequence for Genscan to analyze at once was set to 30 kb. To determine which of these Genscan predicted cDNA sequences encode G-protein coupled receptors, the encoded polypeptides were analyzed by querying against PFAM models for G-protein coupled receptors. Potential G-protein  
35 coupled receptors were also identified by homology to Incyte cDNA sequences that had been

annotated as G-protein coupled receptors. These selected Genscan-predicted sequences were then compared by BLAST analysis to the genpept and gbpr public databases. Where necessary, the Genscan-predicted sequences were then edited by comparison to the top BLAST hit from genpept to correct errors in the sequence predicted by Genscan, such as extra or omitted exons. BLAST analysis was also used to find any Incyte cDNA or public cDNA coverage of the Genscan-predicted sequences, thus providing evidence for transcription. When Incyte cDNA coverage was available, this information was used to correct or confirm the Genscan predicted sequence. Full length polynucleotide sequences were obtained by assembling Genscan-predicted coding sequences with Incyte cDNA sequences and/or public cDNA sequences using the assembly process described in Example III. Alternatively, full length polynucleotide sequences were derived entirely from edited or unedited Genscan-predicted coding sequences.

#### **V. Assembly of Genomic Sequence Data with cDNA Sequence Data**

##### **"Stitched" Sequences**

Partial cDNA sequences were extended with exons predicted by the Genscan gene identification program described in Example IV. Partial cDNAs assembled as described in Example III were mapped to genomic DNA and parsed into clusters containing related cDNAs and Genscan exon predictions from one or more genomic sequences. Each cluster was analyzed using an algorithm based on graph theory and dynamic programming to integrate cDNA and genomic information, generating possible splice variants that were subsequently confirmed, edited, or extended to create a full length sequence. Sequence intervals in which the entire length of the interval was present on more than one sequence in the cluster were identified, and intervals thus identified were considered to be equivalent by transitivity. For example, if an interval was present on a cDNA and two genomic sequences, then all three intervals were considered to be equivalent. This process allows unrelated but consecutive genomic sequences to be brought together, bridged by cDNA sequence. Intervals thus identified were then "stitched" together by the stitching algorithm in the order that they appear along their parent sequences to generate the longest possible sequence, as well as sequence variants. Linkages between intervals which proceed along one type of parent sequence (cDNA to cDNA or genomic sequence to genomic sequence) were given preference over linkages which change parent type (cDNA to genomic sequence). The resultant stitched sequences were translated and compared by BLAST analysis to the genpept and gbpr public databases. Incorrect exons predicted by Genscan were corrected by comparison to the top BLAST hit from genpept. Sequences were further extended with additional cDNA sequences, or by inspection of genomic DNA, when necessary.

##### **"Stretched" Sequences**

Partial DNA sequences were extended to full length with an algorithm based on BLAST analysis. First, partial cDNAs assembled as described in Example III were queried against public

databases such as the GenBank primate, rodent, mammalian, vertebrate, and eukaryote databases using the BLAST program. The nearest GenBank protein homolog was then compared by BLAST analysis to either Incyte cDNA sequences or GenScan exon predicted sequences described in Example IV. A chimeric protein was generated by using the resultant high-scoring segment pairs (HSPs) to map the translated sequences onto the GenBank protein homolog. Insertions or deletions may occur in the chimeric protein with respect to the original GenBank protein homolog. The GenBank protein homolog, the chimeric protein, or both were used as probes to search for homologous genomic sequences from the public human genome databases. Partial DNA sequences were therefore "stretched" or extended by the addition of homologous genomic sequences. The resultant stretched sequences were examined to determine whether it contained a complete gene.

#### VI. Chromosomal Mapping of GCREC Encoding Polynucleotides

The sequences which were used to assemble SEQ ID NO:20-38 were compared with sequences from the Incyte LIFESEQ database and public domain databases using BLAST and other implementations of the Smith-Waterman algorithm. Sequences from these databases that matched SEQ ID NO:20-38 were assembled into clusters of contiguous and overlapping sequences using assembly algorithms such as Phrap (Table 7). Radiation hybrid and genetic mapping data available from public resources such as the Stanford Human Genome Center (SHGC), Whitehead Institute for Genome Research (WIGR), and Généthon were used to determine if any of the clustered sequences had been previously mapped. Inclusion of a mapped sequence in a cluster resulted in the assignment of all sequences of that cluster, including its particular SEQ ID NO., to that map location.

Map locations are represented by ranges, or intervals, of human chromosomes. The map position of an interval, in centiMorgans, is measured relative to the terminus of the chromosome's p-arm. (The centiMorgan (cM) is a unit of measurement based on recombination frequencies between chromosomal markers. On average, 1 cM is roughly equivalent to 1 megabase (Mb) of DNA in humans, although this can vary widely due to hot and cold spots of recombination.) The cM distances are based on genetic markers mapped by Généthon which provide boundaries for radiation hybrid markers whose sequences were included in each of the clusters. Human genome maps and other resources available to the public, such as the NCBI "GeneMap'99" World Wide Web site (<http://www.ncbi.nlm.nih.gov/genemap/>), can be employed to determine if previously identified disease genes map within or in proximity to the intervals indicated above.

#### VII. Analysis of Polynucleotide Expression

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; Ausubel (1995) *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST were used to search for identical or related molecules in cDNA databases such as GenBank or LIFESEQ (Incyte Genomics). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar. The basis of the search is the product score, which is defined as:

$$\frac{\text{BLAST Score} \times \text{Percent Identity}}{5 \times \text{minimum} \{ \text{length}(\text{Seq. 1}), \text{length}(\text{Seq. 2}) \}}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. The product score is a normalized value between 0 and 100, and is calculated as follows: the BLAST score is multiplied by the percent nucleotide identity and the product is divided by (5 times the length of the shorter of the two sequences). The BLAST score is calculated by assigning a score of +5 for every base that matches in a high-scoring segment pair (HSP), and -4 for every mismatch. Two sequences may share more than one HSP (separated by gaps). If there is more than one HSP, then the pair with the highest BLAST score is used to calculate the product score. The product score represents a balance between fractional overlap and quality in a BLAST alignment. For example, a product score of 100 is produced only for 100% identity over the entire length of the shorter of the two sequences being compared. A product score of 70 is produced either by 100% identity and 70% overlap at one end, or by 88% identity and 100% overlap at the other. A product score of 50 is produced either by 100% identity and 50% overlap at one end, or 79% identity and 100% overlap.

Alternatively, polynucleotide sequences encoding GCREC are analyzed with respect to the tissue sources from which they were derived. For example, some full length sequences are assembled, at least in part, with overlapping Incyte cDNA sequences (see Example III). Each cDNA sequence is derived from a cDNA library constructed from a human tissue. Each human tissue is classified into one of the following organ/tissue categories: cardiovascular system; connective tissue; digestive system; embryonic structures; endocrine system; exocrine glands; genitalia, female; genitalia, male; germ cells; hemic and immune system; liver; musculoskeletal system; nervous system; pancreas; respiratory system; sense organs; skin; stomatognathic system; unclassified/mixed; or urinary tract. The number of libraries in each category is counted and divided by the total number of libraries across all categories. Similarly, each human tissue is classified into one of the following disease/condition categories: cancer, cell line, developmental, inflammation, neurological, trauma, cardiovascular, pooled, and other, and the number of libraries in each category is counted and divided by the total number of libraries across all categories. The resulting percentages reflect the tissue- and

disease-specific expression of cDNA encoding GCRC. cDNA sequences and cDNA library/tissue information are found in the LIFESEQ GOLD database (Incyte Genomics, Palo Alto CA).

### VIII. Extension of GCRC Encoding Polynucleotides

Full length polynucleotide sequences were also produced by extension of an appropriate  
5 fragment of the full length molecule using oligonucleotide primers designed from this fragment. One primer was synthesized to initiate 5' extension of the known fragment, and the other primer was synthesized to initiate 3' extension of the known fragment. The initial primers were designed using OLIGO 4.06 software (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target  
10 sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries were used to extend the sequence. If more than one extension was necessary or desired, additional or nested sets of primers were designed.

High fidelity amplification was obtained by PCR using methods well known in the art. PCR  
15 was performed in 96-well plates using the PTC-200 thermal cycler (MJ Research, Inc.). The reaction mix contained DNA template, 200 nmol of each primer, reaction buffer containing  $Mg^{2+}$ ,  $(NH_4)_2SO_4$ , and 2-mercaptoethanol, Taq DNA polymerase (Amersham Pharmacia Biotech), ELONGASE enzyme (Life Technologies), and Pfu DNA polymerase (Stratagene), with the following parameters for primer pair PCI A and PCI B: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C. In the  
20 alternative, the parameters for primer pair T7 and SK+ were as follows: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 57°C, 1 min; Step 4: 68°C, 2 min; Step 5: Steps 2, 3, and 4 repeated 20 times; Step 6: 68°C, 5 min; Step 7: storage at 4°C.

The concentration of DNA in each well was determined by dispensing 100  $\mu$ l PICOGREEN  
25 quantitation reagent (0.25% (v/v) PICOGREEN; Molecular Probes, Eugene OR) dissolved in 1X TE and 0.5  $\mu$ l of undiluted PCR product into each well of an opaque fluorimeter plate (Corning Costar, Acton MA), allowing the DNA to bind to the reagent. The plate was scanned in a Fluoroskan II (Labsystems Oy, Helsinki, Finland) to measure the fluorescence of the sample and to quantify the concentration of DNA. A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by  
30 electrophoresis on a 1 % agarose gel to determine which reactions were successful in extending the sequence.

The extended nucleotides were desalted and concentrated, transferred to 384-well plates, digested with CviJI cholera virus endonuclease (Molecular Biology Research, Madison WI), and sonicated or sheared prior to religation into pUC 18 vector (Amersham Pharmacia Biotech). For  
35 shotgun sequencing, the digested nucleotides were separated on low concentration (0.6 to 0.8%)

agarose gels, fragments were excised, and agar digested with Agar ACE (Promega). Extended clones were religated using T4 ligase (New England Biolabs, Beverly MA) into pUC 18 vector (Amersham Pharmacia Biotech), treated with Pfu DNA polymerase (Stratagene) to fill-in restriction site overhangs, and transfected into competent *E. coli* cells. Transformed cells were selected on antibiotic-containing media, and individual colonies were picked and cultured overnight at 37°C in 384-well plates in LB/2x carb liquid media.

The cells were lysed, and DNA was amplified by PCR using Taq DNA polymerase (Amersham Pharmacia Biotech) and Pfu DNA polymerase (Stratagene) with the following parameters: Step 1: 94°C, 3 min; Step 2: 94°C, 15 sec; Step 3: 60°C, 1 min; Step 4: 72°C, 2 min; Step 5: steps 2, 3, and 4 repeated 29 times; Step 6: 72°C, 5 min; Step 7: storage at 4°C. DNA was quantified by PICOGREEN reagent (Molecular Probes) as described above. Samples with low DNA recoveries were reamplified using the same conditions as described above. Samples were diluted with 20% dimethylsulfoxide (1:2, v/v), and sequenced using DYENAMIC energy transfer sequencing primers and the DYENAMIC DIRECT kit (Amersham Pharmacia Biotech) or the ABI PRISM BIGDYE Terminator cycle sequencing ready reaction kit (Applied Biosystems).

In like manner, full length polynucleotide sequences are verified using the above procedure or are used to obtain 5' regulatory sequences using the above procedure along with oligonucleotides designed for such extension, and an appropriate genomic library.

#### **IX. Labeling and Use of Individual Hybridization Probes**

Hybridization probes derived from SEQ ID NO:20-38 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250 µCi of [ $\gamma$ -<sup>32</sup>P] adenosine triphosphate (Amersham Pharmacia Biotech), and T4 polynucleotide kinase (DuPont NEN, Boston MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size exclusion dextran bead column (Amersham Pharmacia Biotech). An aliquot containing 10<sup>7</sup> counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: Ase I, Bgl II, Eco RI, Pst I, Xba I, or Pvu II (DuPont NEN).

The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under conditions of up to, for example, 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. Hybridization patterns are visualized using autoradiography or an alternative imaging means and



compared.

#### X. Microarrays

The linkage or synthesis of array elements upon a microarray can be achieved utilizing photolithography, piezoelectric printing (ink-jet printing, See, e.g., Baldeschweiler, supra), mechanical microspotting technologies, and derivatives thereof. The substrate in each of the  
5   aforementioned technologies should be uniform and solid with a non-porous surface (Schna (1999), supra). Suggested substrates include silicon, silica, glass slides, glass chips, and silicon wafers. Alternatively, a procedure analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding  
10   procedures. A typical array may be produced using available methods and machines well known to those of ordinary skill in the art and may contain any appropriate number of elements. (See, e.g., Schna, M. et al. (1995) Science 270:467-470; Shalon, D. et al. (1996) Genome Res. 6:639-645; Marshall, A. and J. Hodgson (1998) Nat. Biotechnol. 16:27-31.)

Full length cDNAs, Expressed Sequence Tags (ESTs), or fragments or oligomers thereof may  
15   comprise the elements of the microarray. Fragments or oligomers suitable for hybridization can be selected using software well known in the art such as LASERGENE software (DNASTAR). The array elements are hybridized with polynucleotides in a biological sample. The polynucleotides in the biological sample are conjugated to a fluorescent label or other molecular tag for ease of detection. After hybridization, nonhybridized nucleotides from the biological sample are removed, and a  
20   fluorescence scanner is used to detect hybridization at each array element. Alternatively, laser desorption and mass spectrometry may be used for detection of hybridization. The degree of complementarity and the relative abundance of each polynucleotide which hybridizes to an element on the microarray may be assessed. In one embodiment, microarray preparation and usage is described in detail below.

#### 25   Tissue or Cell Sample Preparation

Total RNA is isolated from tissue samples using the guanidinium thiocyanate method and poly(A)<sup>+</sup> RNA is purified using the oligo-(dT) cellulose method. Each poly(A)<sup>+</sup> RNA sample is reverse transcribed using MMLV reverse-transcriptase, 0.05 pg/ $\mu$ l oligo-(dT) primer (21mer), 1X first strand buffer, 0.03 units/ $\mu$ l RNase inhibitor, 500  $\mu$ M dATP, 500  $\mu$ M dGTP, 500  $\mu$ M dTTP, 40  
30    $\mu$ M dCTP, 40  $\mu$ M dCTP-Cy3 (BDS) or dCTP-Cy5 (Amersham Pharmacia Biotech). The reverse transcription reaction is performed in a 25 ml volume containing 200 ng poly(A)<sup>+</sup> RNA with GEMBRIGHT kits (Incyte). Specific control poly(A)<sup>+</sup> RNAs are synthesized by in vitro transcription from non-coding yeast genomic DNA. After incubation at 37°C for 2 hr, each reaction sample (one with Cy3 and another with Cy5 labeling) is treated with 2.5 ml of 0.5M sodium hydroxide and  
35   incubated for 20 minutes at 85°C to stop the reaction and degrade the RNA. Samples are purified

using two successive CHROMA SPIN 30 gel filtration spin columns (CLONTECH Laboratories, Inc. (CLONTECH), Palo Alto CA) and after combining, both reaction samples are ethanol precipitated using 1 ml of glycogen (1 mg/ml), 60 ml sodium acetate, and 300 ml of 100% ethanol. The sample is then dried to completion using a SpeedVAC (Savant Instruments Inc., Holbrook NY) and

5 resuspended in 14  $\mu$ l 5X SSC/0.2% SDS.

#### Microarray Preparation

Sequences of the present invention are used to generate array elements. Each array element is amplified from bacterial cells containing vectors with cloned cDNA inserts. PCR amplification uses primers complementary to the vector sequences flanking the cDNA insert. Array elements are  
10 amplified in thirty cycles of PCR from an initial quantity of 1-2 ng to a final quantity greater than 5  $\mu$ g. Amplified array elements are then purified using SEPHACRYL-400 (Amersham Pharmacia Biotech).

Purified array elements are immobilized on polymer-coated glass slides. Glass microscope slides (Corning) are cleaned by ultrasound in 0.1% SDS and acetone, with extensive distilled water  
15 washes between and after treatments. Glass slides are etched in 4% hydrofluoric acid (VWR Scientific Products Corporation (VWR), West Chester PA), washed extensively in distilled water, and coated with 0.05% aminopropyl silane (Sigma) in 95% ethanol. Coated slides are cured in a 110°C oven.

Array elements are applied to the coated glass substrate using a procedure described in US  
20 Patent No. 5,807,522, incorporated herein by reference. 1  $\mu$ l of the array element DNA, at an average concentration of 100 ng/ $\mu$ l, is loaded into the open capillary printing element by a high-speed robotic apparatus. The apparatus then deposits about 5 nl of array element sample per slide.

Microarrays are UV-crosslinked using a STRATALINKER UV-crosslinker (Stratagene). Microarrays are washed at room temperature once in 0.2% SDS and three times in distilled water.  
25 Non-specific binding sites are blocked by incubation of microarrays in 0.2% casein in phosphate buffered saline (PBS) (Tropix, Inc., Bedford MA) for 30 minutes at 60°C followed by washes in 0.2% SDS and distilled water as before.

#### Hybridization

Hybridization reactions contain 9  $\mu$ l of sample mixture consisting of 0.2  $\mu$ g each of Cy3 and  
30 Cy5 labeled cDNA synthesis products in 5X SSC, 0.2% SDS hybridization buffer. The sample mixture is heated to 65°C for 5 minutes and is aliquoted onto the microarray surface and covered with an 1.8 cm<sup>2</sup> coverslip. The arrays are transferred to a waterproof chamber having a cavity just slightly larger than a microscope slide. The chamber is kept at 100% humidity internally by the addition of 140  $\mu$ l of 5X SSC in a corner of the chamber. The chamber containing the arrays is

incubated for about 6.5 hours at 60°C. The arrays are washed for 10 min at 45°C in a first wash buffer (1X SSC, 0.1% SDS), three times for 10 minutes each at 45°C in a second wash buffer (0.1X SSC), and dried.

#### Detection

5 Reporter-labeled hybridization complexes are detected with a microscope equipped with an Innova 70 mixed gas 10 W laser (Coherent, Inc., Santa Clara CA) capable of generating spectral lines at 488 nm for excitation of Cy3 and at 632 nm for excitation of Cy5. The excitation laser light is focused on the array using a 20X microscope objective (Nikon, Inc., Melville NY). The slide containing the array is placed on a computer-controlled X-Y stage on the microscope and raster-scanned past the objective. The 1.8 cm x 1.8 cm array used in the present example is scanned with a  
10 resolution of 20 micrometers.

In two separate scans, a mixed gas multiline laser excites the two fluorophores sequentially. Emitted light is split, based on wavelength, into two photomultiplier tube detectors (PMT R1477, Hamamatsu Photonics Systems, Bridgewater NJ) corresponding to the two fluorophores. Appropriate  
15 filters positioned between the array and the photomultiplier tubes are used to filter the signals. The emission maxima of the fluorophores used are 565 nm for Cy3 and 650 nm for Cy5. Each array is typically scanned twice, one scan per fluorophore using the appropriate filters at the laser source, although the apparatus is capable of recording the spectra from both fluorophores simultaneously.

The sensitivity of the scans is typically calibrated using the signal intensity generated by a  
20 cDNA control species added to the sample mixture at a known concentration. A specific location on the array contains a complementary DNA sequence, allowing the intensity of the signal at that location to be correlated with a weight ratio of hybridizing species of 1:100,000. When two samples from different sources (e.g., representing test and control cells), each labeled with a different fluorophore, are hybridized to a single array for the purpose of identifying genes that are  
25 differentially expressed, the calibration is done by labeling samples of the calibrating cDNA with the two fluorophores and adding identical amounts of each to the hybridization mixture.

The output of the photomultiplier tube is digitized using a 12-bit RTI-835H analog-to-digital (A/D) conversion board (Analog Devices, Inc., Norwood MA) installed in an IBM-compatible PC computer. The digitized data are displayed as an image where the signal intensity is mapped using a  
30 linear 20-color transformation to a pseudocolor scale ranging from blue (low signal) to red (high signal). The data is also analyzed quantitatively. Where two different fluorophores are excited and measured simultaneously, the data are first corrected for optical crosstalk (due to overlapping emission spectra) between the fluorophores using each fluorophore's emission spectrum.

A grid is superimposed over the fluorescence signal image such that the signal from each

spot is centered in each element of the grid. The fluorescence signal within each element is then integrated to obtain a numerical value corresponding to the average intensity of the signal. The software used for signal analysis is the GEMTOOLS gene expression analysis program (Incyte).

#### XI. Complementary Polynucleotides

- 5 Sequences complementary to the GCREC-encoding sequences, or any parts thereof, are used to detect, decrease, or inhibit expression of naturally occurring GCREC. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software (National Biosciences) and the coding sequence of GCREC.
- 10 To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the GCREC-encoding transcript.

#### XII. Expression of GCREC

- 15 Expression and purification of GCREC is achieved using bacterial or virus-based expression systems. For expression of GCREC in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac* (*tac*) hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory
- 20 element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express GCREC upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of GCREC in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is
- 25 replaced with cDNA encoding GCREC by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E.K.
- 30 et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, GCREC is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-

35 kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on

immobilized glutathione under conditions that maintain protein activity and antigenicity (Amersham Pharmacia Biotech). Following purification, the GST moiety can be proteolytically cleaved from GCREC at specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN). Methods for protein expression and purification are discussed in Ausubel (1995, supra, ch. 10 and 16). Purified GCREC obtained by these methods can be used directly in the assays shown in Examples XVI, XVII, and XVIII, where applicable.

### XIII. Functional Assays

GCREC function is assessed by expressing the sequences encoding GCREC at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include PCMV SPORT (Life Technologies) and PCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are transiently transfected into a human cell line, for example, an endothelial or hematopoietic cell line, using either liposome formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M.G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of GCREC on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding GCREC and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake

Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding GCREC and other genes of interest can be analyzed by northern analysis or microarray techniques.

#### XIV. Production of GCREC Specific Antibodies

5 GCREC substantially purified using polyacrylamide gel electrophoresis (PAGE; see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the GCREC amino acid sequence is analyzed using LASERGENE software (DNASTAR) to determine regions of high immunogenicity, and a corresponding oligopeptide is  
10 synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel, 1995, *supra*, ch. 11.)

Typically, oligopeptides of about 15 residues in length are synthesized using an ABI 431A peptide synthesizer (Applied Biosystems) using Fmoc chemistry and coupled to KLH (Sigma-  
15 Aldrich, St. Louis MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel, 1995, *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide and anti-GCREC activity by, for example, binding the peptide or GCREC to a substrate, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat  
20 anti-rabbit IgG.

#### XV. Purification of Naturally Occurring GCREC Using Specific Antibodies

Naturally occurring or recombinant GCREC is substantially purified by immunoaffinity chromatography using antibodies specific for GCREC. An immunoaffinity column is constructed by covalently coupling anti-GCREC antibody to an activated chromatographic resin, such as  
25 CNBr-activated SEPHAROSE (Amersham Pharmacia Biotech). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

Media containing GCREC are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of GCREC (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt  
30 antibody/GCREC binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and GCREC is collected.

#### XVI. Identification of Molecules Which Interact with GCREC

GCREC, or biologically active fragments thereof, are labeled with  $^{125}\text{I}$  Bolton-Hunter reagent. (See, e.g., Bolton A.E. and W.M. Hunter (1973) *Biochem. J.* 133:529-539.) Candidate molecules  
35 previously arrayed in the wells of a multi-well plate are incubated with the labeled GCREC, washed,

and any wells with labeled GCREC complex are assayed. Data obtained using different concentrations of GCREC are used to calculate values for the number, affinity, and association of GCREC with the candidate molecules.

Alternatively, molecules interacting with GCREC are analyzed using the yeast two-hybrid  
5 system as described in Fields, S. and O. Song (1989) *Nature* 340:245-246, or using commercially available kits based on the two-hybrid system, such as the MATCHMAKER system (Clontech).

GCREC may also be used in the PATHCALLING process (CuraGen Corp., New Haven CT) which employs the yeast two-hybrid system in a high-throughput manner to determine all interactions between the proteins encoded by two large libraries of genes (Nandabalan, K. et al. (2000) U.S.  
10 Patent No. 6,057,101).

#### **XVII. Demonstration of GCREC Activity**

An assay for GCREC activity measures the expression of GCREC on the cell surface. cDNA encoding GCREC is transfected into an appropriate mammalian cell line. Cell surface proteins are labeled with biotin as described (de la Fuente, M.A. et al. (1997) *Blood* 90:2398-2405).

15 Immunoprecipitations are performed using GCREC-specific antibodies, and immunoprecipitated samples are analyzed using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting techniques. The ratio of labeled immunoprecipitant to unlabeled immunoprecipitant is proportional to the amount of GCREC expressed on the cell surface.

In the alternative, an assay for GCREC activity is based on a prototypical assay for  
20 ligand/receptor-mediated modulation of cell proliferation. This assay measures the rate of DNA synthesis in Swiss mouse 3T3 cells. A plasmid containing polynucleotides encoding GCREC is added to quiescent 3T3 cultured cells using transfection methods well known in the art. The transiently transfected cells are then incubated in the presence of [<sup>3</sup>H]thymidine, a radioactive DNA precursor molecule. Varying amounts of GCREC ligand are then added to the cultured cells.  
25 Incorporation of [<sup>3</sup>H]thymidine into acid-precipitable DNA is measured over an appropriate time interval using a radioisotope counter, and the amount incorporated is directly proportional to the amount of newly synthesized DNA. A linear dose-response curve over at least a hundred-fold GCREC ligand concentration range is indicative of receptor activity. One unit of activity per milliliter is defined as the concentration of GCREC producing a 50% response level, where 100%  
30 represents maximal incorporation of [<sup>3</sup>H]thymidine into acid-precipitable DNA (McKay, I. and I. Leigh, eds. (1993) Growth Factors: A Practical Approach, Oxford University Press, New York NY, p. 73.)

In a further alternative, the assay for GCREC activity is based upon the ability of GPCR family proteins to modulate G protein-activated second messenger signal transduction pathways (e.g.,  
35 cAMP; Gaudin, P. et al. (1998) *J. Biol. Chem.* 273:4990-4996). A plasmid encoding full length

GCREC is transfected into a mammalian cell line (e.g., Chinese hamster ovary (CHO) or human embryonic kidney (HEK-293) cell lines) using methods well-known in the art. Transfected cells are grown in 12-well trays in culture medium for 48 hours, then the culture medium is discarded, and the attached cells are gently washed with PBS. The cells are then incubated in culture medium with or without ligand for 30 minutes, then the medium is removed and cells lysed by treatment with 1 M perchloric acid. The cAMP levels in the lysate are measured by radioimmunoassay using methods well-known in the art. Changes in the levels of cAMP in the lysate from cells exposed to ligand compared to those without ligand are proportional to the amount of GCREC present in the transfected cells.

To measure changes in inositol phosphate levels, the cells are grown in 24-well plates containing  $1 \times 10^5$  cells/well and incubated with inositol-free media and [ $^3\text{H}$ ]myoinositol, 2  $\mu\text{Ci}$ /well, for 48 hr. The culture medium is removed, and the cells washed with buffer containing 10 mM LiCl followed by addition of ligand. The reaction is stopped by addition of perchloric acid. Inositol phosphates are extracted and separated on Dowex AG1-X8 (Bio-Rad) anion exchange resin, and the total labeled inositol phosphates counted by liquid scintillation. Changes in the levels of labeled inositol phosphate from cells exposed to ligand compared to those without ligand are proportional to the amount of GCREC present in the transfected cells.

#### XVIII. Identification of GCREC Ligands

GCREC is expressed in a eukaryotic cell line such as CHO (Chinese Hamster Ovary) or HEK (Human Embryonic Kidney) 293 which have a good history of GPCR expression and which contain a wide range of G-proteins allowing for functional coupling of the expressed GCREC to downstream effectors. The transformed cells are assayed for activation of the expressed receptors in the presence of candidate ligands. Activity is measured by changes in intracellular second messengers, such as cyclic AMP or  $\text{Ca}^{2+}$ . These may be measured directly using standard methods well known in the art, or by the use of reporter gene assays in which a luminescent protein (e.g. firefly luciferase or green fluorescent protein) is under the transcriptional control of a promoter responsive to the stimulation of protein kinase C by the activated receptor (Milligan, G. et al. (1996) Trends Pharmacol. Sci. 17:235-237). Assay technologies are available for both of these second messenger systems to allow high throughput readout in multi-well plate format, such as the adenylyl cyclase activation FlashPlate Assay (NEN Life Sciences Products), or fluorescent  $\text{Ca}^{2+}$  indicators such as Fluo-4 AM (Molecular Probes) in combination with the FLIPR fluorimetric plate reading system (Molecular Devices). In cases where the physiologically relevant second messenger pathway is not known, GCREC may be coexpressed with the G-proteins  $G_{\alpha 15/16}$  which have been demonstrated to couple to a wide range of G-proteins (Offermanns, S. and M.I. Simon (1995) J. Biol. Chem. 270:15175-15180), in order to funnel the signal transduction of the GCREC through a pathway involving phospholipase C and  $\text{Ca}^{2+}$



mobilization. Alternatively, GCREC may be expressed in engineered yeast systems which lack endogenous GPCRs, thus providing the advantage of a null background for GCREC activation screening. These yeast systems substitute a human GPCR and Ga protein for the corresponding components of the endogenous yeast pheromone receptor pathway. Downstream signaling pathways  
5 are also modified so that the normal yeast response to the signal is converted to positive growth on selective media or to reporter gene expression (Broach, J.R. and J. Thorner (1996) Nature 384 (supp.):14-16). The receptors are screened against putative ligands including known GPCR ligands and other naturally occurring bioactive molecules. Biological extracts from tissues, biological fluids and cell supernatants are also screened.

10

Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with certain embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific  
15 embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

Table 1

Incyte Project ID	Polypeptide SEQ ID NO:	Incyte Polypeptide ID	Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID
7474806	1	7474806CD1	20	7474806CB1
7474840	2	7474840CD1	21	7474840CB1
7475092	3	7475092CD1	22	7475092CB1
7341260	4	7341260CD1	23	7341260CB1
7473911	5	7473911CD1	24	7473911CB1
7474767	6	7474767CD1	25	7474767CB1
7475815	7	7475815CD1	26	7475815CB1
60263275	8	60263275CD1	27	60263275CB1
60203310	9	60203310CD1	28	60203310CB1
7477349	10	7477349CD1	29	7477349CB1
55002225	11	55002225CD1	30	55002225CB1
7475686	12	7475686CD1	31	7475686CB1
7482007	13	7482007CD1	32	7482007CB1
6769042	14	6769042CD1	33	6769042CB1
7476053	15	7476053CD1	34	7476053CB1
7480410	16	7480410CD1	35	7480410CB1
55036418	17	55036418CD1	36	55036418CB1
7481701	18	7481701CD1	37	7481701CB1
7481774	19	7481774CD1	38	7481774CB1

Table 2

Polypeptide SEQ ID NO:	Incyte Polypeptide ID	GenBank ID No:	Probability score	GenBank Homolog
1	7474806CD1	g2707256	4.00E-62	[fl][Meleagris gallopavo] G protein coupled P2Y nucleotide receptor
2	7474840CD1	g11967419	4.00E-65	[fl][Mus musculus] vomeronasal (pheromone) receptor V1RC3
3	7475092CD1	g2992628	2.00E-86	[fl][Homo sapiens] putative seven pass transmembrane protein
4	7341260CD1	g3805932	1.00E-10	[fl][Homo sapiens] putative G-Protein coupled receptor, EDG6
5	7473911CD1	g1055254	2.00E-18	Graler, M.H. et al. (1998) Genomics 53:164-169 [fl][Rattus norvegicus] pheromone receptor VN6
6	7474767CD1	g179985	2.00E-14	Dulac, C. and Axel, R. (1995) Cell 83: 195-206 [fl][Homo sapiens] C-C chemokine receptor type 1
7	7475815CD1	g1055254	1.00E-56	Neote, K. et al. (1993) Cell 72:415-425 [fl][Rattus norvegicus] pheromone receptor VN6
8	60263275CD1	g13183149	0	[fl][Homo sapiens] (AF239764) EGF-like module-containing mucin-like receptor EMR3
9	60203310CD1	g3882981	0	[fl][Rattus norvegicus] calcium- independent alpha-latrotoxin receptor
10	7477349CD1	g6979162	3.00E-11	[fl][Rattus norvegicus] macrophage inflammatory protein-1 alpha receptor
11	55002225CD1	g14164383	0	[fl][Homo sapiens] (AB060151) G protein-coupled receptor
12	7475686CD1	g7248884	1.00E-180	[fl][Mus musculus] G-protein coupled receptor GPR73
13	7482007CD1	g5525078	1.00E-104	Parker, R. et al. (2000) Biochim. Biophys. Acta 1491:369-375 [fl][Rattus norvegicus] seven transmembrane receptor
14	6769042CD1	g4164061	1.70E-59	Abe, J. et al. (1999) J. Biol. Chem. 274:19957-19964 [Bos taurus] latrophilin 3 splice variant abbg FEBS Lett. (1999) 443:348-352

Table 2 (cont.)

Polypeptide SEQ ID NO:	Incyte Polypeptide ID	GenBank ID NO:	Probability score	GenBank Homolog
15	7476053CD1	g310075	1.00E-165	[fl][Rattus norvegicus] serotonin receptor Erlander, M.G. et al. (1993) Proc. Natl. Acad. Sci. U.S.A. 90:3452-3456
16	7480410CD1	g3983382	1.90E-88	[Mus musculus] olfactory receptor E3
17	55036418CD1	g3983398	5.00E-99	[Mus musculus] olfactory receptor G3 Krautwurst, D. et al. (1998) Cell 95:917-926
18	7481701CD1	g12007416	2.00E-67	[fl][Mus musculus] m51 olfactory receptor
19	7481774CD1	g5901478	3.10E-106	[Marmota marmota] olfactory receptor

Table 3

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
1	7474806CD1	339	S111 S15 S189 S260 S336 S46 S84 T163 T204 T255 T36 T80	N35 N69	Transmembrane domains: Y89-T109, F130-F149 7 transmembrane receptor (rhodopsin family): L68-Y324 G-protein coupled receptor BL00237: W116-P155, F224-Y235, T255-T281, S316-I332 Rhodopsin-like GPCR superfamily PR00237: Y53-W77, A86-I107, F130-V152, H166-A187, G216-V239, S260-F284, Y306-I332 G-protein coupled receptor: DM00013 P41231 27-322: F45-S327 P51582 29-322: F45-S327 I55450 20-317: F45-R328 P48042 45-340: Y53-S327 G-protein coupled receptor: PD000009: Y89-F188 Pheromone receptor: PD009900: I59-M329	HMMER HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-DOMO BLAST-PRODOM BLAST-PRODOM
2	7474840CD1	335	S237 S28 S318 T22 T86	N168 N184	Transmembrane domains: H166-L186, M240-Y256, Y299-L319 Putative seven pass transmembrane protein: PD138976: R3-L360	HMMER BLAST-PRODOM
3	7475092CD1	428	S257 S286 S295 S342 S356 S365 S412 S79 T156	N180 N207 N284	Transmembrane Domain: L55-L74 7 transmembrane receptor (rhodopsin family): A31-A250 Rhodopsin-like GPCR superfamily signature PR00237B: A50-L71 PR00237C: P92-V114 PR00237D: S123-L144 PR00237F: Q222-L246 PR00237G: L262-T288	HMMER HMMER-PFAM BLIMPS-PRINTS
4	7341260CD1	330	S123 S185 S323 S5 S78 T219	N154 N4 N76 N93	Transmembrane Domain: I118-A136 PHEROMONE RECEPTOR PD009900: S87-V167, K42-Y90	HMMER BLAST-PRODOM
5	7473911CD1	676	S105 S213 S215 S250 S330 S353 S492 S569 S591 S606 S646 S647 S79 T217 T296 T301 T394 T620	N184		HMMER BLAST-PRODOM

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
6	7474767CD1	372	S214 S289 S351 T145 T223 T299	N11 N318 N353	Signal Peptide: M1-T47 Transmembrane Domains: Y32-S52, I74-P100, I184-Y208 7 transmembrane receptor (rhodopsin family): A43-Y285 G-protein coupled receptors proteins BL00237:M94-PI33, Y221-M247, N277-R293 Rhodopsin-like GPCR superfamily signature PR00237: V28-S52, S62-V83, E108-V130, R144-M165, I184-V207, T226-Y250, S267-R293 G-protein coupled receptors signature: E105-T155 G-PROTEIN COUPLED RECEPTORS DM00013 P32246 28-316:A22-R293 P51677 28-316:F27-R293 P25930 33-319:F27-L300 P34981 19-336:V28-I206, S222-R293 Transmembrane Domain: I216-A234 PHEROMONE RECEPTOR PD009900: K42-S170, I112-K271	SPSCAN HMMER HMMER_PFAM BLIMPS_BLOCKS BLIMPS_PRINTS PROFILES CAN BLAST_DOMO HMMER BLAST_PROD OM
7	7475815CD1	271	S115 S142 S188 S203 S79 T269			

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
8	60263275CD1	611	S257 S308 S494 S578 S580 S588 S598 S63 S81 T21 T211 T258 T307 T406 T75 Y585	N104 N148 N161 N209 N238 N286 N293 N345 N409 N414	Signal Peptide: M1-Q22 Transmembrane Domains: V321-L339, W424-T451, M464-I488, Y540-L560 7 transmembrane receptor (Secretin family): D312-V564 Latrophilin/CL-1-like GPS domain: K259-Q309 EGF-like domain: C30-C76 G-protein coupled receptor BL00649: C378-L403, G430-R454, W465-S494 Secretin-like GPCR superfamily signature PR00249: V317-K341, I380-L403, K423-L448 W465-K490, A539-L560 Type I EGF signature PR00009: K25-F40, E48-Y59 G-protein coupled receptors family 2 signatures: V531-E574 RECEPTOR G PROTEIN COUPLED PD000752: L315-F572 RECEPTOR G PROTEIN COUPLED EGF LIKE PD005428:N176-M304 HORMONE; EMR1; LEUCOCYTE; ANTIGEN; DM05221 A57172 465-886:S183-K596 I37225 347-738:K259-G592 P48960 347-738:K259-G592 G-PROTEIN COUPLED RECEPTORS FAMILY 2 DM00378 I49149 56-425:V314-R573, G9-V56 Aspartic acid and asparagine hydroxylation site: C44-C55	HMMER HMMER HMMER_PPFAM BLIMPS_BLOCKS BLIMPS_PRINTS BLIMPS_PRINTS PROFILES SCAN BLAST_PROD OM BLAST_PROD OM BLAST_DOMO BLAST_DOMO MOTIFS

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
9	60203310CD1	1469	S166 S186 S1214 N464 N549 S187 S261 T1253 N759 N772 S325 S351 S1295 N817 N843 N93 S417 S423 T1398 N932 N1098 S460 S591 T1453 N1179 N1196 S630 S761 T1008 N1251 S791 S824 S1144 S88 S907 S1154 S978 T113 T1181 T181 T199 S1295 T294 T303 T1346 T443 T559 S1458 T658 T672 T731 T803 T842 Y121	N139	Signal peptide: M1-S21 Transmembrane domains: V879-F897, V942-L960, F1020-G1040, F1071-W1090 7 transmembrane receptor (secretin family): D874-V1130 Latrophilin/CLL-like GPS domain: F814-V866 G-protein coupled receptor BL00649: C940-L965, G987-V1011, W1022-A1051, S1113-L1138, C498-T525, G884-I929 Secretin-like GPCR superfamily PR00249: V879-R903, V942-L965, R980-S1005, W1022-V1047, A1105-L1126 EMR1 hormone leucocyte antigen: DM05221 I37225 347-738: L769-S1160 P48960 347-738: L769-S1160 A57172 465-886: N730-T1162 G-protein coupled receptors family 2: DM00378 I49149 56-425: G836-R1131 Latrophilin-related receptor: PD024331: K386-E628 PD041747: L1207-L1469 Myocilin/Olfactomedin response protein: PD006897: Y177-V385 EGF-like G-protein coupled receptor: PD005428: R629-V866 Transmembrane domains: P166-L188, V215-V233 7 transmembrane receptor (rhodopsin family): A251-Y421 G-protein coupled receptor motif: A251-L267 G-protein coupled receptors signature: I243-A293 G-protein coupled receptor BL00237: R231-P270, Y329-N340, P357-V383, H413-R429 Rhodopsin-like GPCR superfamily: I165-A189, S199-A220, E245-L267, R281-W302, K321-I344, S362-Y386, L403-R429 G-protein coupled receptor: DM00013 I49339 28-316: A162-V435 P51675 28-317: A162-V435	HMMER HMMER HMMER-PFAM HMMER-PFAM BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-DOMO BLAST-DOMO BLAST-PRODOM BLAST-PRODOM BLAST-PRODOM HMMER HMMER-PFAM MOTIFS ProfileScan BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-DOMO BLAST-DOMO
10	7477349CD1	469	S122 S199 S25 S312 S447 S85 T110 T195 T207 T282 T315 T39 T427 T431 T465	N139		



Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
11	55002225CD1	335	S190 S30 S62 T11 T110 T150 T189 T320 T330 T66	N10 N17	G-PROTEIN COUPLED RECEPTORS DM00013 P32745 38-329:V32-S310 RECEPTOR COUPLED G-PROTEIN TRANSMEMBRANE GLYCOPROTEIN PHOSPHORYLATION LIPOPROTEIN PALMITATE PROTEIN FAMILY PD000009:R61-Y171 G-protein coupled receptor BL00237:S298-Q314, W99-P138, F206-Y217, Q244-I270 Rhodopsin-like GPCR superfamily signature PR00237:I36-I50, P68-L89, D113-L135, K149-V170, T198-L221, L249-V273, Y288-Q314 Transmembrane domain transmem_domain:T34-F57, V194-I218, M252-L272 7 transmembrane receptor (rhodopsin family) 7tm_1:G51-Y306 G_Protein_Receptor A119-L135 G-protein coupled receptors signature g_protein_receptor.prf:T110-L157 G-PROTEIN COUPLED RECEPTORS DM00013 P49146 44-341:K299-F590 RECEPTOR COUPLED G-PROTEIN TRANSMEMBRANE GLYCOPROTEIN PHOSPHORYLATION LIPOPROTEIN PALMITATE PROTEIN FAMILY PD000009:K328-F437 G-protein coupled receptor BL00237:W367-P406, F478-Y489, R514-F540 Rhodopsin-like GPCR superfamily signature PR00237:R381-I403, T415-Y436, F470-S493, T519-V543, F561-M587, V301-T325, T334-F355. Neuropeptide Y receptor PR01012:R326-I338, R381-A396 Transmembrane domain transmem_domain: I302-F320 7 transmembrane receptor (rhodopsin family) 7tm_1:G316-N575 G_Protein_Receptor V387-I403 G-protein coupled receptors signature g_protein_receptor.prf:N378-I428	BLAST_DOMO BLAST_PRODUM BLIMPS_BLOCKS BLIMPS_PRINTS HMMER HMMER_PFAM MOTIFS PROFILESSCAN BLAST_DOMO BLAST_PRODUM BLIMPS_BLOCKS BLIMPS_PRINTS BLIMPS_PRINTS HMMER HMMER_PFAM MOTIFS PROFILESSCAN
12	7475686CD1	630	S230 S269 S601 S607 T223 T519 T581 T586 T620 T81	N332 N584		

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Software
13	7482007CD1	695	S185 S252 S354 S392 S395 S591 S669 T265 T397 T45 T584 T624 T653	N169 N177 N209 N229 N250 N257 N263 N286 N309 N340 N379 N61 N679 N686	HORMONE; EMRL1; LEUCOCYTE; ANTIGEN; DM05221 I37225 347-738:C349-S688 RECEPTOR TRANSMEMBRANE G-PROTEIN COUPLED GLYCOPROTEIN PRECURSOR SIGNAL TYPE POLYPEPTIDE ALTERNATIVE PD000752:N372-R660 G-protein coupled receptors family 2 proteins. BL00649:C473-I498 Secretin-like GPCR superfamily signature PR00249:Y403-W427, A475-I498 Transmembrane domain transmem_domain:I407-T433, M514-T537, L487-F506, L559-V579 7 transmembrane receptor (Secretin family) 7tm_2:D398-I659	BLAST_DOMO BLAST_PRODUM BLIMPS_BLOCKS BLIMPS_PRINTS HMMER HMMER_PPFAM
14	6769042CD1	633	S108 S262 S275 S381 S589 S612 S616 S70 T251 T355 T517	N153 N235 N260 N263 N292 N366 N41 N604 N61 N78	HORMONE; EMRL1; LEUCOCYTE; ANTIGEN; DM05221 A57172 465-886:G279-S593 RECEPTOR TRANSMEMBRANE G-PROTEIN COUPLED GLYCOPROTEIN PRECURSOR SIGNAL TYPE POLYPEPTIDE ALTERNATIVE PD000752:H321-K579 G-protein coupled receptors family 2 proteins. BL00649:S555-I580, C391-L416 cAMP-type GPCR signature PR00247:Y361-R383, A395-I421, Y433-S451 Secretin-like GPCR superfamily signature PR00249:S327-S351, V393-L416, H431-S456, W473-S498, T517-L537, Q547-L568 Transmembrane domain transmem_domain: C333-L350, I472-T494 7 transmembrane receptor (Secretin family) 7tm_2:Q322-V572 Latrophilin/CL-1-like GPS domain GPS:V265-L315 G-protein coupled receptors family 2 signatures g_protein_recep_f2_2.prf: A474- S593	BLAST_DOMO BLAST_PRODUM BLIMPS_BLOCKS BLIMPS_PRINTS BLIMPS_PRINTS HMMER HMMER_PPFAM HMMER_PPFAM PROFILES SCAN

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
15	7476053CD1	370	S217 S27 S287 T116 T163		Signal peptide: M298-T317 Signal cleavage: M1-A65 Transmembrane domain: F48-L68, M298-L316 7 transmembrane receptor (rhodopsin family) 7tm_1: W69-Y351 G-protein coupled receptor BL00237: R120-H159, Q291-T317, N343-N359 Rhodopsin-like GPCR superfamily signature PR00237: L54-P78, P87-P108, D134-I156, A170-L191, A214-Y237, A296-I320, K333-N359 5-hydroxytryptamine 5B receptor PR00519: A3-P19, E20-P36, P36-V50, E196-R204, R246-V260, V260-V270 5HYDROXYTRYPTAMINE 5B RECEPTOR 5HT5B SEROTONIN GPROTEIN COUPLED TRANSMEMBRANE GLYCOPROTEIN MULTIGENE PD027821: M1-H84 G-PROTEIN COUPLED RECEPTORS DM00013 P31387 46-367: P46-T367 I48231 46-367: P46-T367 P47898 34-354: P46-T367 P20905 156-522: P46-S280	HMMER SPSCAN HMMER HMMER_PFBM BLIMPS_BLOCKS BLIMPS_PRINTS BLIMPS_PRINTS BLAST_PRODUM BLAST_DOMO

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
16	7480410CD1	324	S189 S194 S292 S314 S68	N9	Signal peptide: M1-A39 Transmembrane domains: G26-I50, C203-V217 7 transmembrane receptor (rhodopsin family): A42-Y291 G-protein coupled receptors signature: F103-V147 G-protein coupled receptor motif: G111-V127 G-protein coupled receptor BL00237: T283-A299, K91-P130, L208-Y219, R236-L262 Rhodopsin-like GPCR superfamily PR00237: L27-H51, M60-K81, Y105-V127, M200-L223, P25-L49, K273-A299 Olfactory receptor signature PR00245: M60-K81, F178-D192, F239-G254, V275-L286, S292-L306 G-protein coupled receptor: DM00013 P23275   17-306: H21-G307 A57069   15-304: H21-G307 P30954   29-316: L28-G307 S29709   11-299: P25-G307 Olfactory G-protein coupled receptor: PD000921: F169-M247 Olfactory G-protein coupled receptor: PDI49621: V248-R308	SPScan HMMER HMMER-PFAM ProfileScan MOTIFS BLIMPS-BLOCKS BLIMPS-PRINTS BLIMPS-PRINTS BLAST-DOMO BLAST-PRODROM BLAST-PRODROM

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
17	55036418CD1	315	S194 S22 S292 S68 S8 S88	N6	Signal peptide: M1-A24 Transmembrane domains: L26-L46, C98-M119, F201-A220 7 transmembrane receptor (rhodopsin family): G42-Y291 G-protein coupled receptor motif: S111-I127 G-protein coupled receptors signature: L104-G147 G-protein coupled receptor BL00237: K91-P130, L208-Y219, K236-R262, T283-M299 Rhodopsin-like GPCR superfamily PR00237: V27-Y51, M60-K81, F105-I127, I200-L223, K273-M299 Olfactory receptor signature PR00245: M60-K81, F178-D192, L239-G254, A275-L286, S292-L306 G-protein coupled receptor: DM00013 P23275 17-306: I18-L302 A57069 15-304: F19-K304 P34982 17-305: V27-D307 P23270 18-311: L26-L306 Olfactory G-protein coupled receptor: PD000921: Y169-L246 Olfactory G-protein coupled receptor: PD149621: T247-R308	SPScan HMMER HMMER-PFAM MOTIFS ProfileScan BLIMPS-BLOCKS BLIMPS-PRINTS BLIMPS-PRINTS BLAST-DOMO BLAST-PRODOR BLAST-PRODOR

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
18	7481701CD1	324	S292 S314 S320 S67 S87 T136 T8		Transmembrane domains: L32-N52, V243-F263, Y267-N287 7 transmembrane receptor (rhodopsin family): G41-F180, V225-Y291 Visual pigments (opsins) retinal binding site: W271-T319 G-protein coupled receptor BL00237: R89-P128, R236-R262, S283-K299 Rhodopsin-like GPCR superfamily PR00237: V26-Q50, M59-K80, F103-I125, F12-F33, T144-F167, A238-R262, I273-K299 Olfactory receptor signature PR00245: M59-K80, I176-D190, F239-G254, S292-I306 Melanocortin receptor family PR00534: L51-I63, I125-T136 G-protein coupled receptor: DM00013 P23267 20-309: F17-I306 S29709 11-299: V29-G307 P23270 18-311: F17-K304 P23274 18-306: V26-I302 Olfactory G-protein coupled receptor: PD000921: L165-I247	HMMER HMMER-PFAM ProfileScan BLIMPS-BLOCKS BLIMPS-PRINTS BLIMPS-PRINTS BLIMPS-PRINTS BLAST-DOMO BLAST-PRODOM

Table 3 (cont.)

SEQ ID NO:	Incyte Polypeptide ID	Amino Acid Residues	Potential Phosphorylation Sites	Potential Glycosylation Sites	Signature Sequences, Domains and Motifs	Analytical Methods and Databases
19	7481774CD1	312	S16 S230 S264 S47 S65 T161 T289	N40	Transmembrane domains: I24-V46, Q98-M116, F198-L214 7 transmembrane receptor (rhodopsin family): G39-Y288 G-protein coupled receptor motif: V108-I124 G-protein coupled receptors signature: F100-T146 G-protein coupled receptor BL00237: K88-P127, V205-Y216, Q233-Q259, T280-K296 Olfactory receptor signature PR00245: M57-Q78, F175-S189, F236-G251, L272-L283, T289-L303 G-protein coupled receptor: DM00013 P23275 17-306: S16-L303 A57069 15-304: F15-L303 P34982 17-305: S16-L303 P30953 18-306: R18-L303 Olfactory G-protein coupled receptor: PD149621: T244-R307 Olfactory G-protein coupled receptor: PD000921: C167-L243	HMMER HMMER-PFAM MOTIFS ProfileScan BLIMPS-BLOCKS BLIMPS-PRINTS BLAST-DOMO BLAST-PRODOR BLAST-PRODOR

Table 4

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragment(s)	Sequence Fragments	5' Position	3' Position
20	7474806CB1	1076	1-1076	7075196H1 (BRAUTDR04) g7248967_edit g7407927_edit	1 533 1	532 1076 1102
21	7474840CB1	1102	1007-1102, 107-160, 486-522, 642-842			
22	7475092CB1	2529	628-1475, 1-81	55049853H1 71906055V1 1351856F6 (LATRTUT02) GNN.g7023955_000031_002 71900320V1 8017335J1 (BMARTXE01) 55049805J1 7341260H1 (COLNDIN02) 70811587V1	876 564 1757 1 1988 64 1256 307 1239	1612 1209 2367 468 2529 614 1956 991 1847
23	7341260CB1	1847	1-140, 1490-1847	6834479H1 (BRSTNON02) 70888652V1 7628842H1 (GBLADIE01) FL140044_00001	974 228 1 1	1575 660 284 2031
24	7473911CB1	2031	1-504, 669-1834	GNN.g6693326_000106_002 55093139J1	139 1	1130 276
25	7474767CB1	1130	1-1130	CpG_991027_B15_masked_fa. FL7475815_g8492585_000004_ g3892596	1 372	723 1202
26	7475815CB1	1202	367-959, 1044-1202, 1-116			
27	60263275CB1	2079	134-490, 902-981	71704087V1 71651942V1 3642425T6 (LUNGNOT34) 524802R6 (CARCTXT01) 2435123H1 (BRAVUNT02) 71651560V1	85 986 1469 391 1 838	700 1727 2079 985 255 1544



Table 4

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragment(s)	Sequence Fragments	5' Position	3' Position
28	60203310CB1	5324	1-373, 963-3661, 4111-4162	71959831V1 60203311D1 7638002J1 (SEMVTDE01) 491493H1 (HNT2AGT01) 71957696V1 4028716F7 (BRAINOT23) 8103587H1 (MIXDDIE02) g900324 8195081H2 (BRAIDIR04) 55073390J1 60200671D1 8195081J2 (BRAIDIR04) 55094091J1 55116120H1 70846216V1 GNN.g8140731_000028_002 GNN.g9309533_000030_002 2021568F6 (CONNNOT01) 71243436V1 70844223V1 70845761V1 72398219V1 72374379V1 72373094V1 55049494J1 CpG_SAE300482544.R1 GNN:g6138786_000002_006.ed it 7290466F6 (BRAIFER06) 55049456J1 6925371H1 (FLACFER06) GNN:g9864547_000007_002.ed it 55084155J2 7341368F8 (COLNDIN02) g1507289	4179 3309 2283 3833 4646 4057 620 3652 1 1549 3444 432 1140 2658 1486 265 1 379 1272 872 804 675 971 814 1 1148 1 1576 461 249 472 1017 1 1879	5068 3622 2900 4073 5324 4787 1259 4165 623 2391 3823 1033 2026 3416 1962 1410 379 873 1931 1416 1415 1384 1558 1540 817 1481 1893 2304 1327 797 2103 1790 440 2322
29	7477349CB1	1962	1-1758			
30	55002225CB1	1558	80-1558, 1-49			
31	7475686CB1	2304	1-1212, 1548-2304			
32	7482007CB1	2322	1-628, 1452-1878, 724-1278			

Table 4

Polynucleotide SEQ ID NO:	Incyte Polynucleotide ID	Sequence Length	Selected Fragment(s)	Sequence Fragments	5' Position	3' Position
33	6769042CB1	2366	800-1248, 1-40, 1717, 1369-1717, 218-361, 1821-1861, 1930-2366	7157074H2 (BRAIFEJ02) 72138116D1 7629227H1 (GBLADIE01) 55147608J1	1169 1704 834 1	1797 2366 1341 908
34	7476053CB1	1458	1-1087	GNN.g7630808_000013_022 g3280262	1201 1088	1414 1458
35	7480410CB1	975	1-816, 894- 975	GBI:g9454621.edit 55036194H1 (GPCRDPV02) GNN.g8979559_000011_002	1 166 1	1110 371 975
36	55036418CB1	948	1-162, 193- 948	55036391H1 (GPCRDPV02) GNN.g7239420_000072_008	166 1	371 948
37	7481701CB1	1086	1-1086	GNN.g9795014_000002_002	1	1086
38	7481774CB1	1529	1-963	55143535J1 70822063V1	127 879	918 1529
				7361408F8 (BRAIFE05) 55142634H1	401 1	1051 384

Table 5

Polynucleotide SEQ ID NO:	Incyte Project ID	Representative Library
20	7474806CB1	BRAUTDR04
22	7475092CB1	LATRTUT02
23	7341260CB1	COLNTUT03
24	7473911CB1	BRSTNOT23
27	60263275CB1	EOSITXT01
28	60203310CB1	BRAITUT01
29	7477349CB1	CONNNOT01
31	7475686CB1	BRAIFER06
32	7482007CB1	COLNDIN02
33	6769042CB1	GBLADIE01
38	7481774CB1	BRAIFEE05

Table 6

Library	Vector	Library Description
BRAIFEE05	PCDNA2.1	This 5' biased random primed library was constructed using RNA isolated from brain tissue removed from Caucasian male fetus who was still born with a hypoplastic left heart at 23 weeks' gestation. Serologies were negative.
BRAIFER06	PCDNA2.1	This random primed library was constructed using RNA isolated from brain tissue removed from a Caucasian male fetus who was stillborn with a hypoplastic left heart at 23 weeks' gestation. Serologies were negative.
BRAITUT01	PSPORT1	Library was constructed using RNA isolated from brain tumor tissue removed from a 50-year-old Caucasian female during a frontal lobectomy. Pathology indicated recurrent grade 3 oligoastrocytoma with focal necrosis and extensive calcification. Patient history included a speech disturbance and epilepsy. The patient's brain had also been irradiated with a total dose of 5,082 cGy (Fraction 8). Family history included a brain tumor.
BRAUTDR04	PCDNA2.1	This random primed library was constructed using RNA isolated from pooled striatum, dorsal caudate nucleus, dorsal putamen, and ventral nucleus accumbens tissue removed from a 55-year-old Caucasian female who died from cholangiocarcinoma. Pathology indicated mild meningeal fibrosis predominately over the convexities, scattered axonal spheroids in the white matter of the cingulate cortex and the thalamus, and a few scattered neurofibrillary tangles in the entorhinal cortex and the periaqueductal gray region. Pathology for the associated tumor tissue indicated well-differentiated cholangiocarcinoma of the liver with residual Budd-Chiari syndrome. Patient history included cholangiocarcinoma, post-operative Budd-Chiari syndrome, biliary ascites, hydrothorax, dehydration, malnutrition, oliguria and acute renal failure. Previous surgeries included cholecystectomy and resection of 85% of the liver.
BRSTNOT23	pINCY	Library was constructed using RNA isolated from diseased breast tissue removed from a 35-year-old Caucasian female during a bilateral reduction mammoplasty. Pathology indicated nonproliferative fibrocystic disease. Family history included type II diabetes, atherosclerotic coronary artery disease, acute myocardial infarction, hyperlipidemia, and coronary artery bypass.
COLNDIN02	pINCY	This normalized library was constructed from 4.72 million independent clones from a diseased colon and colon polyp tissue library. Starting RNA was made from pooled cDNA from two donors. cDNA was generated using mRNA isolated from diseased colon tissue removed from the cecum and descending colon of a 16-year-old Caucasian male (donor A) during partial colectomy, temporary ileostomy, and colonoscopy and from diseased colon polyp tissue removed from the cecum of a 67-year-old female (donor B). Pathology indicated innumerable (greater than 100) adenomatous polyps with low-grade dysplasia involving the entire colonic mucosa in the setting of familial polyposis coli (donor A), and a benign cecum polyp (donor B). Pathology for the associated tumor tissue (B) indicated invasive grade 3 adenocarcinoma that arose in tubulovillous adenoma forming a fungating mass in the cecum. The tumor infiltrated just through the muscularis propria. Multiple (2 of 17) regional lymph nodes were involved by metastatic adenocarcinoma. A tubulovillous adenoma and multiple (6) tubular adenomas with low-grade dysplasia were observed in the cecum and ascending colon. Donor A presented with abdominal pain and

Table 6 (cont.)

Library	Vector	Library Description
COLNFTUT03	pINCY	<p>flatulence. The patient was not taking any medications. Family history included benign colon neoplasm in the father and sibling(s); benign hypertension, cerebrovascular disease, breast cancer, uterine cancer, and type II diabetes in the grandparent(s).</p> <p>Library was constructed using RNA isolated from colon tumor tissue obtained from the sigmoid colon of a 62-year-old Caucasian male during a sigmoidectomy and permanent colostomy. Pathology indicated invasive grade 2 adenocarcinoma. One lymph node contained metastasis with extranodal extension. Patient history included hyperlipidemia, cataract disorder, and dermatitis. Family history included benign hypertension, atherosclerotic coronary artery disease, hyperlipidemia, breast cancer, and prostate cancer.</p>
CONNNOT01	pINCY	<p>Library was constructed using RNA isolated from mesentery fat tissue obtained from a 71-year-old Caucasian male during a partial colectomy and permanent colostomy. Family history included atherosclerotic coronary artery disease, myocardial infarction, and extrinsic asthma.</p>
EOSITXT01	pINCY	<p>Library was constructed using RNA isolated from eosinophils stimulated with IL-5.</p>
GBLADIE01	PCDNA2.1	<p>This 5' biased random primed library was constructed using RNA isolated from diseased gallbladder tissue removed from a 55-year-old Caucasian female during laparoscopic cholecystectomy. Pathology indicated chronic cholecystitis and cholelithiasis (greater than 100 stones). The patient presented with cholelithiasis, abdominal pain, and tremors. Patient history included benign hypertension, Morton's neuroma, facial hirsutism, normal delivery, and tobacco abuse in remission. Previous surgeries included total abdominal hysterectomy, bilateral salpingo-oophorectomy, and adenotonsillectomy. Patient medications included Inderal and Premarin. Family history included breast cancer and ALS in the mother; chronic leukemia and ARDS in the father; breast cancer in the sibling(s); and atherosclerotic coronary artery disease in the grandparent(s).</p>
LATRTUT02	pINCY	<p>Library was constructed using RNA isolated from a myxoma removed from the left atrium of a 43-year-old Caucasian male during annuloplasty. Pathology indicated atrial myxoma. Patient history included pulmonary insufficiency, acute myocardial infarction, atherosclerotic coronary artery disease, hyperlipidemia, and tobacco use. Family history included benign hypertension, acute myocardial infarction, atherosclerotic coronary artery disease, and type II diabetes.</p>

Table 7

Program	Description	Reference	Parameter Threshold
ABI FACTURA	A program that removes vector sequences and masks ambiguous bases in nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
ABI/PARACEL FDF	A Fast Data Finder useful in comparing and annotating amino acid or nucleic acid sequences.	Applied Biosystems, Foster City, CA; Paracel Inc., Pasadena, CA.	Mismatch <50%
ABI AutoAssembler	A program that assembles nucleic acid sequences.	Applied Biosystems, Foster City, CA.	
BLAST	A Basic Local Alignment Search Tool useful in sequence similarity search for amino acid and nucleic acid sequences. BLAST includes five functions: blastp, blastn, blastx, tblastn, and tblastx.	Altschul, S.F. et al. (1990) J. Mol. Biol. 215:403-410; Altschul, S.F. et al. (1997) Nucleic Acids Res. 25:3389-3402.	ESTs: Probability value= 1.0E-8 or less <i>Full Length sequences</i> : Probability value= 1.0E-10 or less
FASTA	A Pearson and Lipman algorithm that searches for similarity between a query sequence and a group of sequences of the same type. FASTA comprises at least five functions: fasta, tfasta, fastx, tfastx, and ssearch.	Pearson, W.R. and D.J. Lipman (1988) Proc. Natl. Acad. Sci. USA 85:2444-2448; Pearson, W.R. (1990) Methods Enzymol. 183:63-98; and Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489.	ESTs: fasta E value=1.06E-6 <i>Assembled ESTs</i> : fasta Identity= 95% or greater and Match length=200 bases or greater; fastx E value=1.0E-8 or less <i>Full Length sequences</i> : fastx score=100 or greater
BLIMPS	A BLocks IMProved Searcher that matches a sequence against those in BLOCKS, PRINTS, DOMO, PRODOM, and PFAM databases to search for gene families, sequence homology, and structural fingerprint regions.	Henikoff, S. and J.G. Henikoff (1991) Nucleic Acids Res. 19:6565-6572; Henikoff, J.G. and S. Henikoff (1996) Methods Enzymol. 266:88-105; and Attwood, T.K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.	Probability value= 1.0E-3 or less
HMMER	An algorithm for searching a query sequence against hidden Markov model (HMM)-based databases of protein family consensus sequences, such as PFAM.	Krogh, A. et al. (1994) J. Mol. Biol. 235:1501-1531; Sonnhammer, E.L.L. et al. (1988) Nucleic Acids Res. 26:320-322; Durbin, R. et al. (1998) Our World View, in a Nutshell, Cambridge Univ. Press, pp. 1-350.	PFAM hits: Probability value= 1.0E-3 or less <i>Signal peptide hits</i> : Score= 0 or greater

Table 7 (cont.)

Program	Description	Reference	Parameter Threshold
ProfileScan	An algorithm that searches for structural and sequence motifs in protein sequences that match sequence patterns defined in Prosite.	Gribkov, M. et al. (1988) CABIOS 4:61-66; Gribkov, M. et al. (1989) Methods Enzymol. 183:146-159; Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.	Normalized quality score $\geq$ GCG-specified "HIGH" value for that particular Prosite motif. Generally, score=1.4-2.1.
Phred	A base-calling algorithm that examines automated sequencer traces with high sensitivity and probability.	Ewing, B. et al. (1998) Genome Res. 8:175-185; Ewing, B. and P. Green (1998) Genome Res. 8:186-194.	
Phrap	A Phils Revised Assembly Program including SWAT and CrossMatch, programs based on efficient implementation of the Smith-Waterman algorithm, useful in searching sequence homology and assembling DNA sequences.	Smith, T.F. and M.S. Waterman (1981) Adv. Appl. Math. 2:482-489; Smith, T.F. and M.S. Waterman (1981) J. Mol. Biol. 147:195-197; and Green, P., University of Washington, Seattle, WA.	Score= 120 or greater; Match length= 56 or greater
Consed	A graphical tool for viewing and editing Phrap assemblies.	Gordon, D. et al. (1998) Genome Res. 8:195-202.	
SPScan	A weight matrix analysis program that scans protein sequences for the presence of secretory signal peptides.	Nielson, H. et al. (1997) Protein Engineering 10:1-6; Claverie, J.M. and S. Audic (1997) CABIOS 12:431-439.	Score=3.5 or greater
TMAP	A program that uses weight matrices to delineate transmembrane segments on protein sequences and determine orientation.	Persson, B. and P. Argos (1994) J. Mol. Biol. 237:182-192; Persson, B. and P. Argos (1996) Protein Sci. 5:363-371.	
TMHMMER	A program that uses a hidden Markov model (HMM) to delineate transmembrane segments on protein sequences and determine orientation.	Sonnhammer, E.L. et al. (1998) Proc. Sixth Intl. Conf. on Intelligent Systems for Mol. Biol., Glasgow et al., eds., The Am. Assoc. for Artificial Intelligence Press, Menlo Park, CA, pp. 175-182.	
Motifs	A program that searches amino acid sequences for patterns that matched those defined in Prosite.	Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221; Wisconsin Package Program Manual, version 9, page M51-59, Genetics Computer Group, Madison, WI.	

What is claimed is:

1. An isolated polypeptide selected from the group consisting of:
  - a) a polypeptide comprising an amino acid sequence selected from the group consisting of  
5 SEQ ID NO:1-19,
  - b) a polypeptide comprising a naturally occurring amino acid sequence at least 90% identical to an amino acid sequence selected from the group consisting of SEQ ID NO:1-19,
  - c) a biologically active fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, and  
10 d) an immunogenic fragment of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19.
2. An isolated polypeptide of claim 1 selected from the group consisting of SEQ ID NO:1-  
19.
- 15 3. An isolated polynucleotide encoding a polypeptide of claim 1.
4. An isolated polynucleotide encoding a polypeptide of claim 2.
- 20 5. An isolated polynucleotide of claim 4 selected from the group consisting of SEQ ID NO:20-38.
6. A recombinant polynucleotide comprising a promoter sequence operably linked to a polynucleotide of claim 3.  
25
7. A cell transformed with a recombinant polynucleotide of claim 6.
8. A transgenic organism comprising a recombinant polynucleotide of claim 6.
- 30 9. A method for producing a polypeptide of claim 1, the method comprising:
  - a) culturing a cell under conditions suitable for expression of the polypeptide, wherein said cell is transformed with a recombinant polynucleotide, and said recombinant polynucleotide comprises a promoter sequence operably linked to a polynucleotide encoding the polypeptide of claim 1, and



b) recovering the polypeptide so expressed.

10. An isolated antibody which specifically binds to a polypeptide of claim 1.

5 11. An isolated polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38,

b) a polynucleotide comprising a naturally occurring polynucleotide sequence at least 90% identical to a polynucleotide sequence selected from the group consisting of SEQ ID NO:20-38,

10 c) a polynucleotide complementary to a polynucleotide of a),

d) a polynucleotide complementary to a polynucleotide of b), and

e) an RNA equivalent of a)-d).

12. An isolated polynucleotide comprising at least 60 contiguous nucleotides of a  
15 polynucleotide of claim 11.

13. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

a) hybridizing the sample with a probe comprising at least 20 contiguous nucleotides  
20 comprising a sequence complementary to said target polynucleotide in the sample, and which probe specifically hybridizes to said target polynucleotide, under conditions whereby a hybridization complex is formed between said probe and said target polynucleotide or fragments thereof, and

b) detecting the presence or absence of said hybridization complex, and, optionally, if present, the amount thereof.

25

14. A method of claim 13, wherein the probe comprises at least 60 contiguous nucleotides.

15. A method for detecting a target polynucleotide in a sample, said target polynucleotide having a sequence of a polynucleotide of claim 11, the method comprising:

a) amplifying said target polynucleotide or fragment thereof using polymerase chain reaction  
30 amplification, and

b) detecting the presence or absence of said amplified target polynucleotide or fragment thereof, and, optionally, if present, the amount thereof.

16. A composition comprising a polypeptide of claim 1 and a pharmaceutically acceptable excipient.

17. A composition of claim 16, wherein the polypeptide has an amino acid sequence selected  
5 from the group consisting of SEQ ID NO:1-19.

18. A method for treating a disease or condition associated with decreased expression of functional GCREC, comprising administering to a patient in need of such treatment the composition of claim 16.

10

19. A method for screening a compound for effectiveness as an agonist of a polypeptide of claim 1, the method comprising:

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting agonist activity in the sample.

15

20. A composition comprising an agonist compound identified by a method of claim 19 and a pharmaceutically acceptable excipient.

21. A method for treating a disease or condition associated with decreased expression of  
20 functional GCREC, comprising administering to a patient in need of such treatment a composition of claim 20.

22. A method for screening a compound for effectiveness as an antagonist of a polypeptide of claim 1, the method comprising:

25

- a) exposing a sample comprising a polypeptide of claim 1 to a compound, and
- b) detecting antagonist activity in the sample.

23. A composition comprising an antagonist compound identified by a method of claim 22 and a pharmaceutically acceptable excipient.

30

24. A method for treating a disease or condition associated with overexpression of functional GCREC, comprising administering to a patient in need of such treatment a composition of claim 23.

25. A method of screening for a compound that specifically binds to the polypeptide of claim

1, said method comprising the steps of:

a) combining the polypeptide of claim 1 with at least one test compound under suitable conditions, and

b) detecting binding of the polypeptide of claim 1 to the test compound, thereby identifying a  
5 compound that specifically binds to the polypeptide of claim 1.

26. A method of screening for a compound that modulates the activity of the polypeptide of claim 1, said method comprising:

a) combining the polypeptide of claim 1 with at least one test compound under conditions  
10 permissive for the activity of the polypeptide of claim 1,

b) assessing the activity of the polypeptide of claim 1 in the presence of the test compound, and

c) comparing the activity of the polypeptide of claim 1 in the presence of the test compound with the activity of the polypeptide of claim 1 in the absence of the test compound, wherein a change  
15 in the activity of the polypeptide of claim 1 in the presence of the test compound is indicative of a compound that modulates the activity of the polypeptide of claim 1.

27. A method for screening a compound for effectiveness in altering expression of a target polynucleotide, wherein said target polynucleotide comprises a sequence of claim 5, the method  
20 comprising:

a) exposing a sample comprising the target polynucleotide to a compound, under conditions suitable for the expression of the target polynucleotide,

b) detecting altered expression of the target polynucleotide, and

c) comparing the expression of the target polynucleotide in the presence of varying amounts  
25 of the compound and in the absence of the compound.

28. A method for assessing toxicity of a test compound, said method comprising:

a) treating a biological sample containing nucleic acids with the test compound;

b) hybridizing the nucleic acids of the treated biological sample with a probe comprising at  
30 least 20 contiguous nucleotides of a polynucleotide of claim 11 under conditions whereby a specific hybridization complex is formed between said probe and a target polynucleotide in the biological sample, said target polynucleotide comprising a polynucleotide sequence of a polynucleotide of claim 11 or fragment thereof;

c) quantifying the amount of hybridization complex; and

d) comparing the amount of hybridization complex in the treated biological sample with the  
35

amount of hybridization complex in an untreated biological sample, wherein a difference in the amount of hybridization complex in the treated biological sample is indicative of toxicity of the test compound.

5           29. A diagnostic test for a condition or disease associated with the expression of GCREC in a biological sample comprising the steps of:

          a) combining the biological sample with an antibody of claim 10, under conditions suitable for the antibody to bind the polypeptide and form an antibody:polypeptide complex; and

          b) detecting the complex, wherein the presence of the complex correlates with the presence  
10 of the polypeptide in the biological sample.

          30. The antibody of claim 10, wherein the antibody is:

          a) a chimeric antibody,

          b) a single chain antibody,

15           c) a Fab fragment,

          d) a F(ab')<sub>2</sub> fragment, or

          e) a humanized antibody.

          31. A composition comprising an antibody of claim 10 and an acceptable excipient.  
20

          32. A method of diagnosing a condition or disease associated with the expression of GCREC in a subject, comprising administering to said subject an effective amount of the composition of claim 31.

25           33. A composition of claim 31, wherein the antibody is labeled.

          34. A method of diagnosing a condition or disease associated with the expression of GCREC in a subject, comprising administering to said subject an effective amount of the composition of claim 33.  
30

          35. A method of preparing a polyclonal antibody with the specificity of the antibody of claim 10 comprising:

          a) immunizing an animal with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, or an immunogenic fragment thereof, under conditions to

elicit an antibody response;

b) isolating antibodies from said animal; and

c) screening the isolated antibodies with the polypeptide, thereby identifying a polyclonal antibody which binds specifically to a polypeptide having an amino acid sequence selected from the

5 group consisting of SEQ ID NO:1-19.

36. An antibody produced by a method of claim 35.

37. A composition comprising the antibody of claim 36 and a suitable carrier.

10

38. A method of making a monoclonal antibody with the specificity of the antibody of claim 10 comprising:

a) immunizing an animal with a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19, or an immunogenic fragment thereof, under conditions to

15 elicit an antibody response;

b) isolating antibody producing cells from the animal;

c) fusing the antibody producing cells with immortalized cells to form monoclonal antibody-producing hybridoma cells;

d) culturing the hybridoma cells; and

20 e) isolating from the culture monoclonal antibody which binds specifically to a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19.

39. A monoclonal antibody produced by a method of claim 38.

25 40. A composition comprising the antibody of claim 39 and a suitable carrier.

41. The antibody of claim 10, wherein the antibody is produced by screening a Fab expression library.

30 42. The antibody of claim 10, wherein the antibody is produced by screening a recombinant immunoglobulin library.

43. A method for detecting a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19 in a sample, comprising the steps of:

a) incubating the antibody of claim 10 with a sample under conditions to allow specific binding of the antibody and the polypeptide; and

b) detecting specific binding, wherein specific binding indicates the presence of a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19 in the sample.

44. A method of purifying a polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19 from a sample, the method comprising:

a) incubating the antibody of claim 10 with a sample under conditions to allow specific binding of the antibody and the polypeptide; and

b) separating the antibody from the sample and obtaining the purified polypeptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-19.

45. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:1.

15

46. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:2.

47. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:3.

20

48. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:4.

49. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:5.

25

50. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:6.

51. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:7.

52. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:8.

30

53. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:9.

54. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:10.

55. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:11.

56. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:12.
57. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:13.
- 5 58. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:14.
59. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:15.
60. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:16.
- 10 61. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:17.
62. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:18.
- 15 63. A polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:19.
64. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:20.
- 20 65. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:21.
66. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:22.
- 25 67. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
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68. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
30 NO:24.
69. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
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NO:26.

71. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
5 NO:27.

72. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:28.

10 73. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:29.

74. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:30.

15 75. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:31.

76. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
20 NO:32.

77. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:33.

25 78. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:34.

79. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:35.

30 80. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:36.

81. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID



NO:37.

82. A polynucleotide of claim 11, comprising the polynucleotide sequence of SEQ ID  
NO:38.

<110> INCYTE GENOMICS, INC.

THORNTON, Michael  
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 YAO, Monique G.  
 LU, Yan  
 TRIBOULEY, Catherine M.  
 POLICKY, Jennifer L.  
 KEARNEY, Liam  
 GRAUL, Richard  
 WARREN, Bridget  
 LEE, Ernestine A.  
 DING, Li

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<130> PI-0176 PCT

<140> To Be Assigned

<141> Herewith

<150> 60/221,478; 60/223,268; 60/227,054; 60/231,121; 60/232,243;  
 60/232,691; 60/235,146

151> 2000-07-27; 2000-08-03; 2000-08-21; 2000-09-08; 2000-09-13;  
 2000-09-15; 2000-09-22

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<211> 339

<212> PRT

<213> Homo sapiens

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<221> misc\_feature

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Lys	Val	Asp	Met	Asn	Thr	Ser	Gln	Glu	Gln	Gly	Leu	Cys	Gln	Phe	35	40	45	
Ser	Glu	Lys	Tyr	Lys	Gln	Val	Tyr	Leu	Ser	Leu	Ala	Tyr	Ser	Ile	50	55	60	
Ile	Phe	Ile	Leu	Gly	Leu	Pro	Leu	Asn	Gly	Thr	Val	Leu	Trp	His	65	70	75	
Ser	Trp	Gly	Gln	Thr	Lys	Arg	Trp	Ser	Cys	Ala	Thr	Thr	Tyr	Leu	80	85	90	
Val	Asn	Leu	Met	Val	Ala	Asp	Leu	Leu	Tyr	Val	Leu	Leu	Pro	Phe	95	100	105	
Leu	Ile	Ile	Thr	Tyr	Ser	Leu	Asp	Asp	Arg	Trp	Pro	Phe	Gly	Glu				

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Leu Leu Cys Lys	Leu Val His Phe Leu Phe Tyr Ile Asn Leu Tyr	
125	130	135
Gly Ser Ile Leu	Leu Leu Thr Cys Ile Ser Val His Gln Phe Leu	
140	145	150
Gly Val Trp His	Pro Leu Cys Ser Leu Pro Tyr Arg Thr Arg	
155	160	165
His Ala Trp Leu	Gly Thr Ser Thr Thr Trp Ala Leu Val Val Leu	
170	175	180
Gln Leu Leu Pro	Thr Leu Ala Phe Ser His Thr Asp Tyr Ile Asn	
185	190	195
Gly Gln Met Ile	Trp Tyr Asp Met Thr Ser Gln Glu Asn Phe Asp	
200	205	210
Arg Leu Phe Ala	Tyr Gly Ile Val Leu Thr Leu Ser Gly Phe Leu	
215	220	225
Ser Pro Ser Leu	Val Ile Leu Val Cys Tyr Ser Leu Met Val Arg	
230	235	240
Ser Leu Ile Lys	Pro Glu Glu Asn Leu Met Arg Thr Gly Asn Thr	
245	250	255
Ala Arg Ala Arg	Ser Ile Arg Thr Ile Leu Leu Val Cys Gly Leu	
260	265	270
Phe Thr Leu Cys	Phe Val Pro Phe His Ile Thr Arg Ser Phe Tyr	
275	280	285
Leu Thr Ile Cys	Phe Leu Leu Ser Gln Asp Cys Gln Leu Leu Met	
290	295	300
Ala Pro Ser Val	Ala Tyr Lys Ile Trp Arg Pro Leu Val Ser Val	
305	310	315
Ser Ser Cys Leu	Asn Pro Val Leu Tyr Phe Leu Ser Arg Gly Ala	
320	325	330
Lys Ile Glu Ser	Gly Ser Ser Arg Asn	
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&lt;211&gt; 335

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7474840CD1

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Cys Ala Pro Ala Trp	Ala Thr Glu Arg Asp	Ser Val Ser Lys Lys
20	25	30
Lys Lys Asn Lys Lys	Lys Asn Leu Phe Ser	Gln Ala Thr Ile Gly
35	40	45
Leu Leu Ala Asn Thr	Phe Phe Leu Phe Phe	Asn Ile Phe Ile Phe
50	55	60
Leu Gln Asp Gln Lys	Ser Lys Pro His Asp	Leu Ile Ser Cys Asn
65	70	75
Ser Ala Phe Ile His	Val Val Met Phe Leu	Thr Val Val Asp Ala
80	85	90
Trp Pro Pro Asp Met	Pro Glu Ser Leu His	Leu Gly Asn Glu Phe
95	100	105
Lys Phe Lys Ser Leu	Ser Tyr Ile Asn Arg	Val Arg Met Gly Leu
110	115	120
Cys Ile Cys Asn Ile	Cys Leu Leu Ser Ile	His Gln Ala Asn Thr
125	130	135
Ile Ser Pro Asn Asn	Phe Cys Leu Ala Arg	Leu Lys Gln Lys Phe
140	145	150
Thr Asn Asn Ile Ile	Met Ser Ser Phe Phe	Ser Phe Phe Phe Trp

Ser Ile Asn Leu	155	Ser Phe Ser Tyr Asn	160	Ile Val Phe Phe Thr	165
	170		175		180
Ala Ser Ser Asn	185	Val Thr Gln Asn Ser	190	Leu Pro Lys Gly Ser	195
	200		205		210
Thr Val His Phe	215	Leu Pro Met Lys Ser	220	Phe Met Arg Lys Val	225
	230		235		240
Phe Thr Leu Thr	245	Leu Ser Arg Asp Val	250	Phe Ile Ile Gly Ile	255
	260		265		270
Leu His Ser Ile	275	Ala His Met Val Ile	280	Leu Val Ser Arg His	285
	290		295		300
Thr Gln Ser Gln	305	His Leu His Ser Ile	310	Ser Ile Ser Pro Gln	315
	320		325		330
Phe Pro Glu Lys	335	Arg Ala Ala Gln Thr		Ile Pro Leu Leu Val	
Tyr Cys Leu Val		Met Cys Trp Val Asp		Leu Ile Ile Ser Ser	
Ser Thr Leu Leu		Trp Thr Cys Asn Pro		Val Phe Leu Ser Met	
Asn Leu Val Gly		Asp Val Tyr Ala Thr		Val Val Leu Leu Glu	
Ile Ser Ser Asp		Lys Asn Ile Val Asp		Ile Leu Gln Asn Met	
Ser Ala Ile Lys		Leu			

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&lt;211&gt; 428

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

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&lt;223&gt; Incyte ID No: 7475092CD1

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Gly Arg Glu Pro Ser	20	Thr Pro Gly Gly Gly	25	Ser Gly Gly Gly Gly	30
	25		25		30
Ala Val Ala Ala Ala	35	Ser Gly Ala Ala Val	40	Pro Gly Ser Val Gln	45
	40		40		45
Leu Ala Leu Ser Val	50	Leu His Ala Leu Leu	55	Tyr Ala Ala Leu Phe	60
	55		55		60
Ala Phe Ala Tyr Leu	65	Gln Leu Trp Arg Leu	70	Leu Leu Tyr Arg Glu	75
	70		70		75
Arg Arg Leu Ser Tyr	80	Gln Ser Leu Cys Leu	85	Phe Leu Cys Leu Leu	90
	85		85		90
Trp Ala Ala Leu Arg	95	Thr Thr Leu Phe Ser	100	Ala Ala Phe Ser Leu	105
	100		100		105
Ser Gly Ser Leu Pro	110	Leu Leu Arg Pro Pro	115	Ala His Leu His Phe	120
	115		115		120
Phe Pro His Trp Leu	125	Tyr Cys Phe Pro Ser	130	Cys Leu Gln Phe	135
	130		130		135
Ser Thr Leu Cys Leu	140	Leu Asn Leu Tyr Leu	145	Ala Glu Val Ile Cys	150
	145		145		150
Lys Val Arg Cys Ala	155	Thr Glu Leu Asp Arg	160	His Lys Ile Leu Leu	165
	160		160		165
His Leu Gly Phe Ile	170	Met Ala Ser Leu Leu	175	Phe Leu Val Val Asn	180
	175		175		180
Leu Thr Cys Ala Met	185	Leu Val His Gly Asp	190	Val Pro Glu Asn Gln	195
	190		190		195
Leu Lys Trp Thr Val		Phe Val Arg Ala Leu		Ile Asn Asp Ser Leu	

	200		205		210
Phe Ile Leu Cys	Ala Ile Ser Leu Val	Cys Tyr Ile Cys Lys	Ile		
	215		220		225
Thr Lys Met Ser	Ser Ala Asn Val Tyr	Leu Glu Ser Lys Gly	Met		
	230		235		240
Ser Leu Cys Gln	Thr Val Val Val Gly	Ser Val Val Ile Leu	Leu		
	245		250		255
Tyr Ser Ser Arg	Ala Cys Tyr Asn Leu	Val Val Val Thr Ile	Ser		
	260		265		270
Gln Asp Thr Leu	Glu Ser Pro Phe Asn	Tyr Gly Trp Asp Asn	Leu		
	275		280		285
Ser Asp Lys Ala	His Val Glu Asp Ile	Ser Gly Glu Glu Tyr	Ile		
	290		295		300
Val Phe Gly Met	Val Leu Phe Leu Trp	Glu His Val Pro Ala	Trp		
	305		310		315
Ser Val Val Leu	Phe Phe Arg Ala Gln	Arg Leu Asn Gln Asn	Leu		
	320		325		330
Ala Pro Ala Gly	Met Ile Asn Ser His	Ser Tyr Ser Ser Arg	Ala		
	335		340		345
Tyr Phe Phe Asp	Asn Pro Arg Arg Tyr	Asp Ser Asp Asp Asp	Leu		
	350		355		360
Pro Arg Leu Gly	Ser Ser Arg Glu Gly	Ser Leu Pro Asn Ser	Gln		
	365		370		375
Ser Leu Gly Trp	Tyr Gly Thr Met Thr	Gly Cys Gly Ser Ser	Ser		
	380		385		390
Tyr Thr Val Thr	Pro His Leu Asn Gly	Pro Met Thr Asp Thr	Ala		
	395		400		405
Pro Leu Leu Phe	Thr Cys Ser Asn Leu	Asp Leu Asn Asn His	His		
	410		415		420
Ser Leu Tyr Val	Thr Pro Gln Asn				
	425				

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&lt;211&gt; 330

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

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&lt;223&gt; Incyte ID No: 7341260CD1

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	20	25	30
Ala Asn Leu Leu	Leu Ala Leu Gly Ile	Ala Trp Asp Arg Arg	Leu
	35	40	45
Arg Ser Pro Pro	Ala Gly Cys Phe Phe	Leu Ser Leu Leu Leu	Ala
	50	55	60
Gly Leu Leu Thr	Gly Leu Ala Leu Pro	Thr Leu Pro Gly Leu	Trp
	65	70	75
Asn Gln Ser Arg	Arg Gly Tyr Trp Ser	Cys Leu Leu Val Tyr	Leu
	80	85	90
Ala Pro Asn Phe	Ser Phe Leu Ser Leu	Leu Ala Asn Leu Leu	Leu
	95	100	105
Val His Gly Glu	Arg Tyr Met Ala Val	Leu Arg Pro Leu Gln	Pro
	110	115	120
Pro Gly Ser Ile	Arg Leu Ala Leu Leu	Leu Thr Trp Ala Gly	Pro
	125	130	135
Leu Leu Phe Ala	Ser Leu Pro Ala Leu	Gly Trp Asn His Trp	Thr
	140	145	150
Pro Gly Ala Asn	Cys Ser Ser Gln Ala	Ile Phe Pro Ala Pro	Tyr

155	160	165
Leu Tyr Leu Glu Val Tyr Gly Leu Leu	Leu Pro Ala Val Gly Ala	
170	175	180
Ala Ala Phe Leu Ser Val Arg Val Leu	Ala Thr Ala His Arg Gln	
185	190	195
Leu Gln Asp Ile Cys Arg Leu Glu Arg	Ala Val Cys Arg Asp Glu	
200	205	210
Pro Ser Ala Leu Ala Arg Ala Leu Thr	Trp Arg Gln Ala Arg Ala	
215	220	225
Gln Ala Gly Ala Met Leu Leu Phe Gly	Leu Cys Trp Gly Pro Tyr	
230	235	240
Val Ala Thr Leu Leu Leu Ser Val Leu	Ala Tyr Glu Gln Arg Pro	
245	250	255
Pro Leu Gly Pro Gly Thr Leu Leu Ser	Leu Leu Ser Leu Gly Ser	
260	265	270
Ala Ser Ala Ala Val Pro Val Ala Met	Gly Leu Gly Asp Gln	
275	280	285
Arg Tyr Thr Ala Pro Trp Arg Ala Ala	Ala Gln Arg Cys Leu Gln	
290	295	300
Gly Leu Trp Gly Arg Ala Ser Arg Asp	Ser Pro Gly Pro Ser Ile	
305	310	315
Ala Tyr His Pro Ser Ser Gln Ser Ser	Val Asp Leu Asp Leu Asn	
320	325	330

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&lt;211&gt; 676

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

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&lt;223&gt; Incyte ID No: 7473911CD1

&lt;400&gt; 5

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20	25	30
Leu Leu Leu Phe His Ile Leu Thr Cys	Leu Leu Lys His Arg Thr	
35	40	45
Lys Pro Ala Asp Leu Ile Val Cys His	Val Ala Leu Ile His Ile	
50	55	60
Ile Leu Leu Leu Pro Thr Glu Phe Ile	Ala Thr Asp Ile Phe Gly	
65	70	75
Ser Gln Asp Ser Glu Asp Asp Ile Lys	His Lys Ser Val Ile Tyr	
80	85	90
Arg Arg Asn Arg Gln Ser Gln His Phe	His Ser Thr Asn Leu Ser	
95	100	105
Pro Lys Ala Pro Pro Glu Lys Met Ala	Thr Gln Thr Ile Leu Leu	
110	115	120
Leu Val Ser Cys Phe Val Ile Val Tyr	Val Leu Asp Cys Val Val	
125	130	135
Ala Ser Cys Ser Gly Leu Val Trp Asn	Ser Asp Pro Val Arg His	
140	145	150
Arg Val Gln Met Leu Val Asp Asn Gly	Tyr Ala Thr Ile Ser Pro	
155	160	165
Ser Val Leu Pro Arg Leu Thr Ala Pro	Asn Glu Trp Arg Ala Ser	
170	175	180
Val Tyr Leu Asn Asp Ser Leu Asn Lys	Cys Ser Asn Gly Arg Leu	
185	190	195
Leu Cys Val Asp Arg Gly Leu Asp Glu	Gly Pro Arg Ser Val Pro	
200	205	210

Lys	Cys	Ser	Glu	Ser	Glu	Thr	Asp	Glu	Asp	Tyr	Ile	Val	Leu	Arg
				215					220					225
Ala	Pro	Leu	Arg	Glu	Asp	Glu	Pro	Lys	Asp	Gly	Gly	Ser	Val	Gly
				230					235					240
Asn	Ala	Ala	Leu	Val	Ser	Pro	Glu	Ala	Ser	Ala	Glu	Glu	Glu	Glu
				245					250					255
Glu	Arg	Glu	Glu	Gly	Gly	Glu	Ala	Cys	Gly	Leu	Glu	Arg	Thr	Gly
				260					265					270
Ala	Gly	Gly	Glu	Gln	Val	Asp	Leu	Gly	Glu	Leu	Pro	Asp	His	Glu
				275					280					285
Glu	Lys	Ser	Asn	Gln	Lys	Val	Ala	Ala	Ala	Thr	Leu	Glu	Asp	Arg
				290					295					300
Thr	Gln	Asp	Glu	Pro	Ala	Glu	Glu	Ser	Cys	Gln	Ile	Val	Leu	Phe
				305					310					315
Gln	Asn	Asn	Cys	Met	Asp	Asn	Phe	Val	Thr	Ser	Leu	Thr	Gly	Ser
				320					325					330
Pro	Tyr	Glu	Phe	Phe	Pro	Thr	Lys	Ser	Thr	Ser	Phe	Cys	Arg	Glu
				335					340					345
Ser	Cys	Ser	Pro	Phe	Ser	Glu	Ser	Val	Lys	Ser	Leu	Glu	Ser	Glu
				350					355					360
Gln	Ala	Pro	Lys	Leu	Gly	Leu	Cys	Ala	Glu	Glu	Asp	Pro	Val	Val
				365					370					375
Gly	Ala	Leu	Cys	Gly	Gln	His	Gly	Pro	Leu	Gln	Asp	Gly	Val	Ala
				380					385					390
Glu	Gly	Pro	Thr	Ala	Pro	Asp	Val	Val	Val	Leu	Pro	Lys	Glu	Glu
				395					400					405
Glu	Lys	Glu	Glu	Val	Ile	Val	Asp	Asp	Met	Leu	Ala	Asn	Pro	Tyr
				410					415					420
Val	Met	Gly	Asp	Glu	Gly	Glu	Glu	Glu	Glu	Glu	Glu	Phe	Val	Asp
				425					430					435
Asp	Thr	Leu	Ala	Asn	Pro	Tyr	Val	Met	Gly	Val	Gly	Leu	Pro	Gly
				440					445					450
Arg	Gly	Gly	Glu	Glu	Glu	Glu	Glu	Glu	Glu	Val	Val	Asp	Asp	Thr
				455					460					465
Leu	Ala	Ser	Leu	Tyr	Lys	Met	Gly	Glu	Glu	His	Arg	His	Lys	Gly
				470					475					480
Leu	Ala	Pro	Leu	Trp	Glu	Gly	Gly	Gln	Lys	Pro	Ser	Gln	Lys	Leu
				485					490					495
Pro	Pro	Lys	Lys	Pro	Asp	Leu	Arg	Gln	Val	Pro	Gln	Pro	Leu	Ala
				500					505					510
Ser	Glu	Val	Pro	Gln	Arg	Arg	Gln	Glu	Arg	Ala	Val	Val	Thr	Glu
				515					520					525
Gly	Arg	Pro	Leu	Glu	Ala	Ser	Arg	Ala	Leu	Pro	Ala	Lys	Pro	Arg
				530					535					540
Ala	Phe	Thr	Leu	Tyr	Pro	Arg	Ser	Phe	Ser	Val	Glu	Gly	Gln	Glu
				545					550					555
Ile	Pro	Val	Ser	Ile	Ser	Val	Tyr	Trp	Glu	Pro	Glu	Gly	Ser	Gly
				560					565					570
Leu	Asp	Asp	His	Arg	Ile	Lys	Arg	Lys	Glu	Glu	His	Leu	Ser	Val
				575					580					585
Val	Ser	Gly	Ser	Phe	Ser	Gln	Arg	Asn	His	Leu	Pro	Ser	Ser	Gly
				590					595					600
Thr	Ser	Thr	Pro	Ser	Ser	Met	Val	Asp	Ile	Pro	Pro	Pro	Phe	Asp
				605					610					615
Leu	Ala	Cys	Ile	Thr	Lys	Lys	Pro	Ile	Thr	Lys	Ser	Ser	Pro	Ser
				620					625					630
Leu	Leu	Ile	Asp	Ser	Asp	Ser	Pro	Asp	Lys	Tyr	Lys	Lys	Lys	Lys
				635					640					645
Ser	Ser	Phe	Lys	Arg	Phe	Leu	Ala	Leu	Met	Phe	Asn	Lys	Met	Glu
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Arg	Pro	Gly	Thr	Met	Ala	His	Ala	Cys	His	Pro	Ser	Thr	Leu	Gly
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Ser														

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 35 40 45  
 Leu Thr Val Ile Ile Leu Ser Gln Leu Val Ala Arg Arg Gln Lys  
 50 55 60  
 Ser Ser Tyr Asn Tyr Leu Leu Ala Leu Ala Ala Asp Ile Leu  
 65 70 75  
 Val Leu Phe Phe Ile Val Phe Val Asp Phe Leu Leu Glu Asp Phe  
 80 85 90  
 Ile Leu Asn Met Gln Met Pro Gln Val Pro Asp Lys Ile Ile Glu  
 95 100 105  
 Val Leu Glu Phe Ser Ser Ile His Thr Ser Ile Trp Ile Thr Val  
 110 115 120  
 Pro Leu Thr Ile Asp Arg Tyr Ile Ala Val Cys His Pro Leu Lys  
 125 130 135  
 Tyr His Thr Val Ser Tyr Pro Ala Arg Thr Arg Lys Val Ile Val  
 140 145 150  
 Ser Val Tyr Ile Thr Cys Phe Leu Thr Ser Ile Pro Tyr Tyr Trp  
 155 160 165  
 Trp Pro Asn Ile Trp Thr Glu Asp Tyr Ile Ser Thr Ser Val His  
 170 175 180  
 His Val Leu Ile Trp Ile His Cys Phe Thr Val Tyr Leu Val Pro  
 185 190 195  
 Cys Ser Ile Phe Phe Ile Leu Asn Ser Ile Ile Val Tyr Lys Leu  
 200 205 210  
 Arg Arg Lys Ser Asn Phe Arg Leu Arg Gly Tyr Ser Thr Gly Lys  
 215 220 225  
 Thr Thr Ala Ile Leu Phe Thr Ile Thr Ser Ile Phe Ala Thr Leu  
 230 235 240  
 Trp Ala Pro Arg Ile Ile Met Ile Leu Tyr His Leu Tyr Gly Ala  
 245 250 255  
 Pro Ile Gln Asn Arg Trp Leu Val His Ile Met Ser Asp Ile Ala  
 260 265 270  
 Asn Met Leu Ala Leu Leu Asn Thr Ala Ile Asn Phe Phe Leu Tyr  
 275 280 285  
 Cys Phe Ile Ser Lys Arg Phe Arg Thr Met Ala Ala Ala Thr Leu  
 290 295 300  
 Lys Ala Phe Phe Lys Cys Gln Lys Gln Pro Val Gln Phe Tyr Thr  
 305 310 315  
 Asn His Asn Phe Ser Ile Thr Ser Ser Pro Trp Ile Ser Pro Ala  
 320 325 330  
 Asn Ser His Cys Ile Lys Met Leu Val Tyr Gln Tyr Asp Lys Asn  
 335 340 345  
 Gly Lys Pro Ile Lys Ser Arg Asn Asp Ser Lys Ser Ser Tyr Gln  
 350 355 360  
 Phe Glu Asp Ala Ile Gly Ala Cys Val Ile Ile Leu  
 365 370



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Ala	Ala	Phe	Ser	Glu	Val	Gly	Ile	Gly	Ile	Ser	Ala	Asn	Ala	Met	
			20						25					30	
Leu	Leu	Leu	Phe	His	Ile	Leu	Thr	Cys	Leu	Leu	Lys	His	Arg	Thr	
			35						40					45	
Lys	Pro	Ala	Asp	Leu	Ile	Val	Cys	His	Val	Ala	Leu	Ile	His	Ile	
			50						55					60	
Ile	Leu	Leu	Leu	Pro	Thr	Glu	Phe	Ile	Ala	Thr	Asp	Ile	Phe	Gly	
			65						70					75	
Ser	Gln	Asp	Ser	Glu	Asp	Asp	Ile	Lys	His	Lys	Ser	Val	Ile	Tyr	
			80						85					90	
Arg	Tyr	Arg	Leu	Met	Arg	Gly	Leu	Ser	Ile	Ser	Thr	Thr	Cys	Leu	
			95						100					105	
Leu	Ser	Ile	Leu	Pro	Ala	Ile	Thr	Cys	Ser	Pro	Arg	Ser	Ser	Cys	
			110						115					120	
Leu	Ala	Val	Phe	Lys	Asp	Ser	His	Ile	Thr	Asn	His	Val	Ala	Phe	
			125						130					135	
Ser	Ser	Val	Phe	His	Ile	Ser	Ile	Ser	Asp	Ser	Phe	Leu	Val	Ser	
			140						145					150	
Thr	Leu	Pro	Ile	Lys	Asn	Leu	Ala	Ser	Asn	Ser	Leu	Thr	Phe	Val	
			155						160					165	
Thr	Gln	Ser	Cys	Ser	Ala	Gly	Ile	Gly	Ser	Arg	Pro	Pro	Ser	Ser	
			170						175					180	
Gly	Tyr	Met	Val	Ile	Leu	Leu	Ser	Arg	Arg	Asn	Arg	Gln	Ser	Gln	
			185						190					195	
His	Phe	His	Ser	Thr	Asn	Leu	Ser	Pro	Lys	Ala	Pro	Pro	Glu	Lys	
			200						205					210	
Met	Ala	Thr	Gln	Thr	Ile	Leu	Leu	Leu	Val	Ser	Cys	Phe	Val	Ile	
			215						220					225	
Val	Tyr	Val	Leu	Asp	Cys	Val	Val	Ala	Ser	Cys	Ser	Gly	Leu	Val	
			230						235					240	
Trp	Asn	Ser	Asp	Pro	Val	Arg	His	Arg	Val	Gln	Met	Leu	Val	Asp	
			245						250					255	
Asn	Gly	Tyr	Ala	Thr	Ile	Ser	Pro	Ser	Val	Leu	Val	Ser	Thr	Glu	
			260						265					270	

Lys

<210> 8  
 <211> 611  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 60263275CD1

<400> 8

Met	Gln	Gly	Pro	Leu	Leu	Leu	Pro	Gly	Leu	Cys	Phe	Leu	Leu	Ser	
1				5					10					15	
Leu	Phe	Gly	Ala	Val	Thr	Gln	Lys	Thr	Lys	Asn	Ile	Asn	Glu	Cys	
			20						25					30	

Thr	Pro	Pro	Tyr	Ser	Val	Tyr	Cys	Gly	Phe	Asn	Ala	Val	Cys	Tyr	
				35					40					45	
Asn	Val	Glu	Gly	Ser	Phe	Tyr	Cys	Gln	Cys	Val	Pro	Gly	Tyr	Arg	
				50					55					60	
Leu	His	Ser	Gly	Asn	Glu	Gln	Phe	Ser	Asn	Ser	Asn	Glu	Asn	Thr	
				65					70					75	
Cys	Gln	Asp	Thr	Thr	Ser	Ser	Lys	Thr	Thr	Gln	Gly	Arg	Lys	Glu	
				80					85					90	
Leu	Gln	Lys	Ile	Val	Asp	Lys	Phe	Glu	Ser	Leu	Leu	Thr	Asn	Gln	
				95					100					105	
Thr	Leu	Trp	Arg	Thr	Glu	Gly	Arg	Gln	Glu	Ile	Ser	Ser	Thr	Ala	
				110					115					120	
Thr	Thr	Ile	Leu	Arg	Asp	Val	Glu	Ser	Lys	Val	Leu	Glu	Thr	Ala	
				125					130					135	
Leu	Lys	Asp	Pro	Glu	Gln	Lys	Val	Leu	Lys	Ile	Gln	Asn	Asp	Ser	
				140					145					150	
Val	Ala	Ile	Glu	Thr	Gln	Ala	Ile	Thr	Asp	Asn	Cys	Ser	Glu	Glu	
				155					160					165	
Arg	Lys	Thr	Phe	Asn	Leu	Asn	Val	Gln	Met	Asn	Ser	Met	Asp	Ile	
				170					175					180	
Arg	Cys	Ser	Asp	Ile	Ile	Gln	Gly	Asp	Thr	Gln	Gly	Pro	Ser	Ala	
				185					190					195	
Ile	Ala	Phe	Ile	Ser	Tyr	Ser	Ser	Leu	Gly	Asn	Ile	Ile	Asn	Ala	
				200					205					210	
Thr	Phe	Phe	Glu	Glu	Met	Asp	Lys	Lys	Asp	Gln	Val	Tyr	Leu	Asn	
				215					220					225	
Ser	Gln	Val	Val	Ser	Ala	Ala	Ile	Gly	Pro	Lys	Arg	Asn	Val	Ser	
				230					235					240	
Leu	Ser	Lys	Ser	Val	Thr	Leu	Thr	Phe	Gln	His	Val	Lys	Met	Thr	
				245					250					255	
Pro	Ser	Thr	Lys	Lys	Val	Phe	Cys	Val	Tyr	Trp	Lys	Ser	Thr	Gly	
				260					265					270	
Gln	Gly	Ser	Gln	Trp	Ser	Arg	Asp	Gly	Cys	Phe	Leu	Ile	His	Val	
				275					280					285	
Asn	Lys	Ser	His	Thr	Met	Cys	Asn	Cys	Ser	His	Leu	Ser	Ser	Phe	
				290					295					300	
Ala	Val	Leu	Met	Ala	Leu	Thr	Ser	Gln	Glu	Glu	Asp	Pro	Val	Leu	
				305					310					315	
Thr	Val	Ile	Thr	Tyr	Val	Gly	Leu	Ser	Val	Ser	Leu	Leu	Cys	Leu	
				320					325					330	
Leu	Leu	Ala	Ala	Leu	Thr	Phe	Leu	Leu	Cys	Lys	Ala	Ile	Gln	Asn	
				335					340					345	
Thr	Ser	Thr	Ser	Leu	His	Leu	Gln	Leu	Ser	Leu	Cys	Leu	Phe	Leu	
				350					355					360	
Ala	His	Leu	Leu	Phe	Leu	Val	Gly	Ile	Asp	Arg	Thr	Glu	Pro	Lys	
				365					370					375	
Val	Leu	Cys	Ser	Ile	Ile	Ala	Gly	Ala	Leu	His	Tyr	Leu	Tyr	Leu	
				380					385					390	
Ala	Ala	Phe	Thr	Trp	Met	Leu	Leu	Glu	Gly	Val	His	Leu	Phe	Leu	
				395					400					405	
Thr	Ala	Arg	Asn	Leu	Thr	Val	Val	Asn	Tyr	Ser	Ser	Ile	Asn	Arg	
				410					415					420	
Leu	Met	Lys	Trp	Ile	Met	Phe	Pro	Val	Gly	Tyr	Gly	Val	Pro	Ala	
				425					430					435	
Val	Thr	Val	Ala	Ile	Ser	Ala	Ala	Ser	Trp	Pro	His	Leu	Tyr	Gly	
				440					445					450	
Thr	Ala	Asp	Arg	Cys	Trp	Leu	His	Leu	Asp	Gln	Gly	Phe	Met	Trp	
				455					460					465	
Ser	Phe	Leu	Gly	Pro	Val	Cys	Ala	Ile	Phe	Ser	Ala	Asn	Leu	Val	
				470					475					480	
Leu	Phe	Ile	Leu	Val	Phe	Trp	Ile	Leu	Lys	Arg	Lys	Leu	Ser	Ser	
				485					490					495	
Leu	Asn	Ser	Glu	Val	Ser	Thr	Ile	Gln	Asn	Thr	Arg	Met	Leu	Ala	

	500		505		510
Phe Lys Ala Thr	Ala Gln Leu Phe Ile	Leu Gly Cys Thr Trp Cys			
	515	520		525	
Leu Gly Leu Leu	Gln Val Gly Pro Ala	Ala Gln Val Met Ala Tyr			
	530	535		540	
Leu Phe Thr Ile	Ile Asn Ser Leu Gln	Gly Phe Phe Ile Phe Leu			
	545	550		555	
Val Tyr Cys Leu	Leu Ser Gln Gln Val	Gln Lys Gln Tyr Gln Lys			
	560	565		570	
Trp Phe Arg Glu	Ile Val Lys Ser Lys	Ser Glu Ser Glu Thr Tyr			
	575	580		585	
Thr Leu Ser Ser	Lys Met Gly Pro Asp	Ser Lys Pro Ser Glu Gly			
	590	595		600	
Asp Val Phe Pro	Gly Gln Val Lys Arg	Lys Tyr			
	605	610			

&lt;210&gt; 9

&lt;211&gt; 1469

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 60203310CD1

&lt;400&gt; 9

Met Trp Pro Ser Gln	Leu Leu Ile Phe	Met Met Leu Leu Ala Pro	
1	5	10	15
Ile Ile His Ala Phe	Ser Arg Ala Pro	Ile Pro Met Ala Val Val	
	20	25	30
Arg Arg Glu Leu Ser	Cys Glu Ser Tyr	Pro Ile Glu Leu Arg Cys	
	35	40	45
Pro Gly Thr Asp Val	Ile Met Ile Glu	Ser Ala Asn Tyr Gly Arg	
	50	55	60
Thr Asp Asp Lys Ile	Cys Asp Ser Asp	Pro Ala Gln Met Glu Asn	
	65	70	75
Ile Arg Cys Tyr Leu	Pro Asp Ala Tyr	Lys Ile Met Ser Gln Arg	
	80	85	90
Cys Asn Asn Arg Thr	Gln Cys Ala Val	Val Ala Gly Pro Asp Val	
	95	100	105
Phe Pro Asp Pro Cys	Pro Gly Thr Tyr	Lys Tyr Leu Glu Val Gln	
	110	115	120
Tyr Glu Cys Val Pro	Tyr Lys Val Glu	Gln Lys Val Phe Leu Cys	
	125	130	135
Pro Gly Leu Leu Lys	Gly Val Tyr Gln	Ser Glu His Leu Phe Glu	
	140	145	150
Ser Asp His Gln Ser	Gly Ala Trp Cys	Lys Asp Pro Leu Gln Ala	
	155	160	165
Ser Asp Lys Ile Tyr	Tyr Met Pro Trp	Thr Pro Tyr Arg Thr Asp	
	170	175	180
Thr Leu Thr Glu Tyr	Ser Ser Lys Asp	Asp Phe Ile Ala Gly Arg	
	185	190	195
Pro Thr Thr Thr Tyr	Lys Leu Pro His	Arg Val Asp Gly Thr Gly	
	200	205	210
Phe Val Val Tyr Asp	Gly Ala Leu Phe	Phe Asn Lys Glu Arg Thr	
	215	220	225
Arg Asn Ile Val Lys	Phe Asp Leu Arg	Thr Arg Ile Lys Ser Gly	
	230	235	240
Glu Ala Ile Ile Ala	Asn Ala Asn Tyr	His Asp Thr Ser Pro Tyr	
	245	250	255
Arg Trp Gly Gly Lys	Ser Asp Ile Asp	Leu Ala Val Asp Glu Asn	
	260	265	270
Gly Leu Trp Val Ile	Tyr Ala Thr Glu	Gln Asn Asn Gly Lys Ile	

	275		280		285
Val Ile Ser Gln	Leu Asn Pro Tyr Thr	Leu Arg Ile Glu Gly Thr			
	290		295		300
Trp Asp Thr Ala	Tyr Asp Lys Arg Ser	Ala Ser Asn Ala Phe Met			
	305		310		315
Ile Cys Gly Ile	Leu Tyr Val Val Lys	Ser Val Tyr Glu Asp Asp			
	320		325		330
Asp Asn Glu Ala	Thr Gly Asn Lys Ile	Asp Tyr Ile Tyr Asn Thr			
	335		340		345
Asp Gln Ser Lys	Asp Ser Leu Val Asp	Val Pro Phe Pro Asn Ser			
	350		355		360
Tyr Gln Tyr Ile	Ala Ala Val Asp Tyr	Asn Pro Arg Asp Asn Leu			
	365		370		375
Leu Tyr Val Trp	Asn Asn Tyr His Val	Val Lys Tyr Ser Leu Asp			
	380		385		390
Phe Gly Pro Leu	Asp Ser Arg Ser Gly	Gln Ala His His Gly Gln			
	395		400		405
Val Ser Tyr Ile	Ser Pro Pro Ile His	Leu Asp Ser Glu Leu Glu			
	410		415		420
Arg Pro Ser Val	Lys Asp Ile Ser Thr	Thr Gly Pro Leu Gly Met			
	425		430		435
Gly Ser Thr Thr	Thr Ser Thr Thr Leu	Arg Thr Thr Thr Leu Ser			
	440		445		450
Pro Gly Arg Ser	Thr Thr Pro Ser Val	Ser Gly Arg Arg Asn Arg			
	455		460		465
Ser Thr Ser Thr	Pro Ser Pro Ala Val	Glu Val Leu Asp Asp Met			
	470		475		480
Thr Thr His Leu	Pro Ser Ala Ser Ser	Gln Ile Pro Ala Leu Glu			
	485		490		495
Glu Ser Cys Glu	Ala Val Glu Ala Arg	Glu Ile Met Trp Phe Lys			
	500		505		510
Thr Arg Gln Gly	Gln Ile Ala Lys Gln	Pro Cys Pro Ala Gly Thr			
	515		520		525
Ile Gly Val Ser	Thr Tyr Leu Cys Leu	Ala Pro Asp Gly Ile Trp			
	530		535		540
Asp Pro Gln Gly	Pro Asp Leu Ser Asn	Cys Ser Ser Pro Trp Val			
	545		550		555
Asn His Ile Thr	Gln Lys Leu Lys Ser	Gly Glu Thr Ala Ala Asn			
	560		565		570
Ile Ala Arg Glu	Leu Ala Glu Gln Thr	Arg Asn His Leu Asn Ala			
	575		580		585
Gly Asp Ile Thr	Tyr Ser Val Arg Ala	Met Asp Gln Leu Val Gly			
	590		595		600
Leu Leu Asp Val	Gln Leu Arg Asn Leu	Thr Pro Gly Gly Lys Asp			
	605		610		615
Ser Ala Ala Arg	Ser Leu Asn Lys Leu	Gln Lys Arg Glu Arg Ser			
	620		625		630
Cys Arg Ala Tyr	Val Gln Ala Met Val	Glu Thr Val Asn Asn Leu			
	635		640		645
Leu Gln Pro Gln	Ala Leu Asn Ala Trp	Arg Asp Leu Thr Thr Ser			
	650		655		660
Asp Gln Leu Arg	Ala Ala Thr Met Leu	Leu His Thr Val Glu Glu			
	665		670		675
Ser Ala Phe Val	Leu Ala Asp Asn Leu	Leu Lys Thr Asp Ile Val			
	680		685		690
Arg Glu Asn Thr	Asp Asn Ile Lys Leu	Glu Val Ala Arg Leu Ser			
	695		700		705
Thr Glu Gly Asn	Leu Glu Asp Leu Lys	Phe Pro Glu Asn Met Gly			
	710		715		720
His Gly Ser Thr	Ile Gln Leu Ser Ala	Asn Thr Leu Lys Gln Asn			
	725		730		735
Gly Arg Asn Gly	Glu Ile Arg Val Ala	Phe Val Leu Tyr Asn Asn			
	740		745		750

Leu	Gly	Pro	Tyr	Leu	Ser	Thr	Glu	Asn	Ala	Ser	Met	Lys	Leu	Gly
				755					760					765
Thr	Glu	Ala	Leu	Ser	Thr	Asn	His	Ser	Val	Ile	Val	Asn	Ser	Pro
				770					775					780
Val	Ile	Thr	Ala	Ala	Ile	Asn	Lys	Glu	Phe	Ser	Asn	Lys	Val	Tyr
				785					790					795
Leu	Ala	Asp	Pro	Val	Val	Phe	Thr	Val	Lys	His	Ile	Lys	Gln	Ser
				800					805					810
Glu	Glu	Asn	Phe	Asn	Pro	Asn	Cys	Ser	Phe	Trp	Ser	Tyr	Ser	Lys
				815					820					825
Arg	Thr	Met	Thr	Gly	Tyr	Trp	Ser	Thr	Gln	Gly	Cys	Arg	Leu	Leu
				830					835					840
Thr	Thr	Asn	Lys	Thr	His	Thr	Thr	Cys	Ser	Cys	Asn	His	Leu	Thr
				845					850					855
Asn	Phe	Ala	Val	Leu	Met	Ala	His	Val	Glu	Val	Lys	His	Ser	Asp
				860					865					870
Ala	Val	His	Asp	Leu	Leu	Leu	Asp	Val	Ile	Thr	Trp	Val	Gly	Ile
				875					880					885
Leu	Leu	Ser	Leu	Val	Cys	Leu	Leu	Ile	Cys	Ile	Phe	Thr	Phe	Cys
				890					895					900
Phe	Phe	Arg	Gly	Leu	Gln	Ser	Asp	Arg	Asn	Thr	Ile	His	Lys	Asn
				905					910					915
Leu	Cys	Ile	Ser	Leu	Phe	Val	Ala	Glu	Leu	Leu	Phe	Leu	Ile	Gly
				920					925					930
Ile	Asn	Arg	Thr	Asp	Gln	Pro	Ile	Ala	Cys	Ala	Val	Phe	Ala	Ala
				935					940					945
Leu	Leu	His	Phe	Phe	Phe	Leu	Ala	Ala	Phe	Thr	Trp	Met	Phe	Leu
				950					955					960
Glu	Gly	Val	Gln	Leu	Tyr	Ile	Met	Leu	Val	Glu	Val	Phe	Glu	Ser
				965					970					975
Glu	His	Ser	Arg	Arg	Lys	Tyr	Phe	Tyr	Leu	Val	Gly	Tyr	Gly	Met
				980					985					990
Pro	Ala	Leu	Ile	Val	Ala	Val	Ser	Ala	Ala	Val	Asp	Tyr	Arg	Ser
				995					1000					1005
Tyr	Gly	Thr	Asp	Lys	Val	Cys	Trp	Leu	Arg	Leu	Asp	Thr	Tyr	Phe
				1010					1015					1020
Ile	Trp	Ser	Phe	Ile	Gly	Pro	Ala	Thr	Leu	Ile	Ile	Met	Leu	Asn
				1025					1030					1035
Val	Ile	Phe	Leu	Gly	Ile	Ala	Leu	Tyr	Lys	Met	Val	His	His	Thr
				1040					1045					1050
Ala	Ile	Leu	Lys	Pro	Glu	Ser	Gly	Cys	Leu	Asp	Asn	Ile	Asn	Tyr
				1055					1060					1065
Glu	Asp	Asn	Arg	Pro	Phe	Ile	Lys	Ser	Trp	Val	Ile	Gly	Ala	Ile
				1070					1075					1080
Ala	Leu	Leu	Cys	Leu	Leu	Gly	Leu	Thr	Trp	Ala	Phe	Gly	Leu	Met
				1085					1090					1095
Tyr	Ile	Asn	Glu	Ser	Thr	Val	Ile	Met	Ala	Tyr	Leu	Phe	Thr	Ile
				1100					1105					1110
Phe	Asn	Ser	Leu	Gln	Gly	Met	Phe	Ile	Phe	Ile	Phe	His	Cys	Val
				1115					1120					1125
Leu	Gln	Lys	Lys	Val	Arg	Lys	Glu	Tyr	Gly	Lys	Cys	Leu	Arg	Thr
				1130					1135					1140
His	Cys	Cys	Ser	Gly	Lys	Ser	Thr	Glu	Ser	Ser	Ile	Gly	Ser	Gly
				1145					1150					1155
Lys	Thr	Ser	Gly	Ser	Arg	Thr	Pro	Gly	Arg	Tyr	Ser	Thr	Gly	Ser
				1160					1165					1170
Gln	Ser	Arg	Ile	Arg	Arg	Met	Trp	Asn	Asp	Thr	Val	Arg	Lys	Gln
				1175					1180					1185
Ser	Glu	Ser	Ser	Phe	Ile	Thr	Gly	Asp	Ile	Asn	Ser	Ser	Ala	Ser
				1190					1195					1200
Leu	Asn	Arg	Glu	Gly	Leu	Leu	Asn	Asn	Ala	Arg	Asp	Thr	Ser	Val
				1205					1210					1215
Met	Asp	Thr	Leu	Pro	Leu	Asn	Gly	Asn	His	Gly	Asn	Ser	Tyr	Ser

1220	1225	1230
Ile Ala Ser Gly Glu Tyr Leu Ser Asn Cys Val Gln Ile Ile Asp		
1235	1240	1245
Arg Gly Tyr Asn His Asn Glu Thr Ala Leu Glu Lys Lys Ile Leu		
1250	1255	1260
Lys Glu Leu Thr Ser Asn Tyr Ile Pro Ser Tyr Leu Asn Asn His		
1265	1270	1275
Glu Arg Ser Ser Glu Gln Asn Arg Asn Leu Met Asn Lys Leu Val		
1280	1285	1290
Asn Asn Leu Gly Ser Gly Arg Glu Asp Asp Ala Ile Val Leu Asp		
1295	1300	1305
Asp Ala Thr Ser Phe Asn His Glu Glu Ser Leu Gly Leu Glu Leu		
1310	1315	1320
Ile His Glu Glu Ser Asp Ala Pro Leu Leu Pro Pro Arg Val Tyr		
1325	1330	1335
Ser Thr Glu Asn His Gln Pro His His Tyr Thr Arg Arg Arg Ile		
1340	1345	1350
Pro Gln Asp His Ser Glu Ser Phe Phe Pro Leu Leu Thr Asn Glu		
1355	1360	1365
His Thr Glu Asp Leu Gln Ser Pro His Arg Asp Ser Leu Tyr Thr		
1370	1375	1380
Ser Met Pro Thr Leu Ala Gly Val Ala Ala Thr Glu Ser Val Thr		
1385	1390	1395
Thr Ser Thr Gln Thr Glu Pro Pro Pro Ala Lys Cys Gly Asp Ala		
1400	1405	1410
Glu Asp Val Tyr Tyr Lys Ser Met Pro Asn Leu Gly Ser Arg Asn		
1415	1420	1425
His Val His Gln Leu His Thr Tyr Tyr Gln Leu Gly Arg Gly Ser		
1430	1435	1440
Ser Asp Gly Phe Ile Val Pro Pro Asn Lys Asp Gly Thr Pro Pro		
1445	1450	1455
Glu Gly Ser Ser Lys Gly Pro Ala His Leu Val Thr Ser Leu		
1460	1465	

&lt;210&gt; 10

&lt;211&gt; 469

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7477349CD1

&lt;400&gt; 10

Met Asp Pro Ser Val	Val Ser Asn Glu Tyr Tyr Asp Val Ala His	
1	5	10 15
Gly Ala Lys Asp Pro	Val Val Pro Thr Ser Leu Gln Asp Ile Thr	
20	25	30
Ala Val Leu Gly Thr	Glu Ala Tyr Thr Glu Glu Asp Lys Ser Met	
35	40	45
Val Ser His Ala Gln	Lys Ser Gln His Ser Cys Leu Ser His Ser	
50	55	60
Arg Trp Leu Arg Ser	Pro Gln Val Thr Gly Gly Ser Trp Asp Leu	
65	70	75
Arg Ile Arg Pro Ser	Lys Asp Ser Ser Ser Phe Arg Gln Ala Gln	
80	85	90
Cys Leu Arg Lys Asp	Pro Gly Ala Asn Asn His Leu Glu Ser Gln	
95	100	105
Gly Val Arg Gly Thr	Ala Gly Asp Ala Asp Arg Glu Leu Arg Gly	
110	115	120
Pro Ser Glu Lys Ala	Thr Ala Gly Gln Pro Arg Val Thr Leu Leu	
125	130	135
Pro Thr Pro Asn Val	Ser Gly Leu Ser Gln Glu Phe Glu Ser His	

Trp	Pro	Glu	Ile	Ala	Glu	Arg	Ser	Pro	Cys	Val	Ala	Gly	Val	Ile	140	145	150
Pro	Val	Ile	Tyr	Tyr	Ser	Val	Leu	Leu	Gly	Leu	Gly	Leu	Pro	Val	155	160	165
Ser	Leu	Leu	Thr	Ala	Val	Ala	Leu	Ala	Arg	Leu	Ala	Thr	Arg	Thr	170	175	180
Arg	Arg	Pro	Ser	Tyr	Tyr	Tyr	Leu	Leu	Ala	Leu	Thr	Ala	Ser	Asp	185	190	195
Ile	Ile	Ile	Gln	Val	Val	Ile	Val	Phe	Ala	Gly	Phe	Leu	Leu	Gln	200	205	210
Gly	Ala	Val	Leu	Ala	Arg	Gln	Val	Pro	Gln	Ala	Val	Val	Arg	Thr	215	220	225
Ala	Asn	Ile	Leu	Glu	Phe	Ala	Ala	Asn	His	Ala	Ser	Val	Trp	Ile	230	235	240
Ala	Ile	Leu	Leu	Thr	Val	Asp	Arg	Tyr	Thr	Ala	Leu	Cys	His	Pro	245	250	255
Leu	His	His	Arg	Ala	Ala	Ser	Ser	Pro	Gly	Arg	Thr	Arg	Arg	Ala	260	265	270
Ile	Ala	Ala	Val	Leu	Ser	Ala	Ala	Leu	Leu	Thr	Gly	Ile	Pro	Phe	275	280	285
Tyr	Trp	Trp	Leu	Asp	Met	Trp	Arg	Asp	Thr	Asp	Ser	Pro	Arg	Thr	290	295	300
Leu	Asp	Glu	Val	Leu	Lys	Trp	Ala	His	Cys	Leu	Thr	Val	Tyr	Phe	305	310	315
Ile	Pro	Cys	Gly	Val	Phe	Leu	Val	Thr	Asn	Ser	Ala	Ile	Ile	His	320	325	330
Arg	Leu	Arg	Arg	Arg	Gly	Arg	Ser	Gly	Leu	Gln	Pro	Arg	Val	Gly	335	340	345
Lys	Ser	Thr	Ala	Ile	Leu	Leu	Gly	Ile	Thr	Thr	Leu	Phe	Thr	Leu	350	355	360
Leu	Trp	Ala	Pro	Arg	Val	Phe	Val	Met	Leu	Tyr	His	Met	Tyr	Val	365	370	375
Ala	Pro	Val	His	Arg	Asp	Trp	Arg	Val	His	Leu	Ala	Leu	Asp	Val	380	385	390
Ala	Asn	Met	Val	Ala	Met	Leu	His	Thr	Ala	Ala	Asn	Phe	Gly	Leu	395	400	405
Tyr	Cys	Phe	Val	Ser	Lys	Thr	Phe	Arg	Ala	Thr	Val	Arg	Gln	Val	410	415	420
Ile	His	Asp	Ala	Tyr	Leu	Pro	Cys	Thr	Leu	Ala	Ser	Gln	Pro	Glu	425	430	435
Gly	Met	Ala	Ala	Lys	Pro	Val	Met	Glu	Pro	Pro	Gly	Leu	Pro	Thr	440	445	450
Gly	Ala	Glu	Val												455	460	465

&lt;210&gt; 11

&lt;211&gt; 335

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 55002225CD1

&lt;400&gt; 11

Met	Asn	Pro	Phe	His	Ala	Ser	Cys	Trp	Asn	Thr	Ser	Ala	Glu	Leu	1	5	10	15
Leu	Asn	Lys	Ser	Trp	Asn	Lys	Glu	Phe	Ala	Tyr	Gln	Thr	Ala	Ser	20	25	30	35
Val	Val	Asp	Thr	Val	Ile	Leu	Pro	Ser	Met	Ile	Gly	Ile	Ile	Cys	40	45		
Ser	Thr	Gly	Leu	Val	Gly	Asn	Ile	Leu	Ile	Val	Phe	Thr	Ile	Ile				

	50		55		60
Arg Ser Arg Lys Lys Thr Val Pro Asp Ile Tyr Ile Cys Asn Leu					
	65		70		75
Ala Val Ala Asp Leu Val His Ile Val Gly Met Pro Phe Leu Ile					
	80		85		90
His Gln Trp Ala Arg Gly Gly Glu Trp Val Phe Gly Gly Pro Leu					
	95		100		105
Cys Thr Ile Ile Thr Ser Leu Asp Thr Cys Asn Gln Phe Ala Cys					
	110		115		120
Ser Ala Ile Met Thr Val Met Ser Val Asp Arg Tyr Phe Ala Leu					
	125		130		135
Val Gln Pro Phe Arg Leu Thr Arg Trp Arg Thr Arg Tyr Lys Thr					
	140		145		150
Ile Arg Ile Asn Leu Gly Leu Trp Ala Ala Ser Phe Ile Leu Ala					
	155		160		165
Leu Pro Val Trp Val Tyr Ser Lys Val Ile Lys Phe Lys Asp Gly					
	170		175		180
Val Glu Ser Cys Ala Phe Asp Leu Thr Ser Pro Asp Asp Val Leu					
	185		190		195
Trp Tyr Thr Leu Tyr Leu Thr Ile Thr Thr Phe Phe Phe Pro Leu					
	200		205		210
Pro Leu Ile Leu Val Cys Tyr Ile Leu Ile Leu Cys Tyr Thr Trp					
	215		220		225
Glu Met Tyr Gln Gln Asn Lys Asp Ala Arg Cys Cys Asn Pro Ser					
	230		235		240
Val Pro Lys Gln Arg Val Met Lys Leu Thr Lys Met Val Leu Val					
	245		250		255
Leu Val Val Val Phe Ile Leu Ser Ala Ala Pro Tyr His Val Ile					
	260		265		270
Gln Leu Val Asn Leu Gln Met Glu Gln Pro Thr Leu Ala Phe Tyr					
	275		280		285
Val Gly Tyr Tyr Leu Ser Ile Cys Leu Ser Tyr Ala Ser Ser Ser					
	290		295		300
Ile Asn Pro Phe Leu Tyr Ile Leu Leu Ser Gly Thr Pro Gln Ile					
	305		310		315
Gln Arg Arg Ala Thr Glu Lys Glu Ile Asn Asn Met Gly Asn Thr					
	320		325		330
Leu Lys Ser His Phe					
	335				

&lt;210&gt; 12

&lt;211&gt; 630

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7475686CD1

&lt;400&gt; 12

Met Arg Leu Gly Pro Val Pro Ala Arg Ala Arg Ala Leu Leu Ser					
1	5		10		15
Trp Val Arg Gly Leu Glu Ser Arg Gly Gly Glu Trp Thr Lys Cys					
	20		25		30
Ile Val Gln Leu Gly His Leu Leu Ala Thr Gln His Pro Ala Ala					
	35		40		45
Pro Thr Cys Gly Val Val Ser Ser Ala Leu Val Met His Ser Thr					
	50		55		60
Asp Val Cys Leu Ala Pro Thr Met His Gln Ala Leu Asp Trp Ala					
	65		70		75
Ala Gly Ile Trp Phe Thr Gly Arg Leu Gly Leu Arg Glu His Lys					
	80		85		90
Ser Leu Ala Gln Gly Asp Ser Val Cys Pro Cys Glu Ser Glu Leu					



				95					100					105
Gly	Asp	Phe	Gln	Val	Tyr	Gly	Leu	Val	Ser	Thr	Glu	Gly	Val	Val
				110					115					120
Ser	Cys	Phe	Gly	Glu	Lys	Thr	Pro	Gln	His	Pro	Gly	Pro	Pro	Ala
				125					130					135
Ser	Leu	Ser	Leu	Ala	Asn	Arg	Cys	His	Asn	Val	Val	Thr	Ala	Val
				140					145					150
Gly	Ala	Trp	Pro	Ala	His	Gly	Ser	Ile	Leu	Gly	Asn	Val	Pro	Glu
				155					160					165
Ala	Pro	Val	Gly	Ala	Asp	Val	Leu	Gly	Ala	Gly	Gly	Cys	Asp	Trp
				170					175					180
Ala	Asp	Lys	Glu	Ala	Leu	Ala	Pro	Gly	Gln	Arg	Ala	Lys	Val	His
				185					190					195
Ile	Leu	Leu	Glu	Ser	Ser	Gly	Gln	Ser	Asp	Pro	Ser	Tyr	Ala	Val
				200					205					210
Leu	Pro	Asp	Ser	Trp	Ala	Ala	Thr	Glu	Gly	Phe	Pro	Thr	Tyr	Arg
				215					220					225
Ser	Gln	Val	Ser	Ser	Pro	Arg	Ile	Pro	Gly	Ser	Ser	Ile	Trp	Leu
				230					235					240
Gly	Ser	Gly	Ser	Gly	Trp	Pro	Ile	Leu	Gly	Glu	Leu	Arg	Glu	Cys
				245					250					255
Asp	Gln	Met	Phe	Ser	Cys	Met	Leu	Pro	Thr	Gly	Cys	Ala	Ser	Phe
				260					265					270
Gln	Asp	Pro	Gly	Arg	Tyr	Gly	Asp	Tyr	Asp	Leu	Pro	Met	Asp	Glu
				275					280					285
Asp	Glu	Asp	Met	Thr	Lys	Thr	Arg	Thr	Phe	Phe	Ala	Ala	Lys	Ile
				290					295					300
Val	Ile	Gly	Ile	Ala	Leu	Ala	Gly	Ile	Met	Leu	Val	Cys	Gly	Ile
				305					310					315
Gly	Asn	Phe	Val	Phe	Ile	Ala	Ala	Leu	Thr	Arg	Tyr	Lys	Lys	Leu
				320					325					330
Arg	Asn	Leu	Thr	Asn	Leu	Leu	Ile	Ala	Asn	Leu	Ala	Ile	Ser	Asp
				335					340					345
Phe	Leu	Val	Ala	Ile	Ile	Cys	Cys	Pro	Phe	Glu	Met	Asp	Tyr	Tyr
				350					355					360
Val	Val	Arg	Gln	Leu	Ser	Trp	Glu	His	Gly	His	Val	Leu	Cys	Ala
				365					370					375
Ser	Val	Asn	Tyr	Leu	Arg	Thr	Val	Ser	Leu	Tyr	Val	Ser	Thr	Asn
				380					385					390
Ala	Leu	Leu	Ala	Ile	Ala	Ile	Asp	Arg	Tyr	Leu	Ala	Ile	Val	His
				395					400					405
Pro	Leu	Lys	Pro	Arg	Met	Asn	Tyr	Gln	Thr	Ala	Ser	Phe	Leu	Ile
				410					415					420
Ala	Leu	Val	Trp	Met	Val	Ser	Ile	Leu	Ile	Ala	Ile	Pro	Ser	Ala
				425					430					435
Tyr	Phe	Ala	Thr	Glu	Thr	Val	Leu	Phe	Ile	Val	Lys	Ser	Gln	Glu
				440					445					450
Lys	Ile	Phe	Cys	Gly	Gln	Ile	Trp	Pro	Val	Asp	Gln	Gln	Leu	Tyr
				455					460					465
Tyr	Lys	Ser	Tyr	Phe	Leu	Phe	Ile	Phe	Gly	Val	Glu	Phe	Val	Gly
				470					475					480
Pro	Val	Val	Thr	Met	Thr	Leu	Cys	Tyr	Ala	Arg	Ile	Ser	Arg	Glu
				485					490					495
Leu	Trp	Phe	Lys	Ala	Val	Pro	Gly	Phe	Gln	Thr	Glu	Gln	Ile	Arg
				500					505					510
Lys	Arg	Leu	Arg	Cys	Arg	Arg	Lys	Thr	Val	Leu	Val	Leu	Met	Cys
				515					520					525
Ile	Leu	Thr	Ala	Tyr	Val	Leu	Cys	Trp	Ala	Pro	Phe	Tyr	Gly	Phe
				530					535					540
Thr	Ile	Val	Arg	Asp	Phe	Phe	Pro	Thr	Val	Phe	Val	Lys	Glu	Lys
				545					550					555
His	Tyr	Leu	Thr	Ala	Phe	Tyr	Val	Val	Glu	Cys	Ile	Ala	Met	Ser
				560					565					570

Asn	Ser	Met	Ile	Asn	Thr	Val	Cys	Phe	Val	Thr	Val	Lys	Asn	Asn
				575					580					585
Thr	Met	Lys	Tyr	Phe	Lys	Lys	Met	Met	Leu	Leu	His	Trp	Arg	Pro
				590					595					600
Ser	Gln	Arg	Gly	Ser	Lys	Ser	Ser	Ala	Asp	Leu	Asp	Leu	Arg	Thr
				605					610					615
Asn	Gly	Val	Pro	Thr	Thr	Glu	Glu	Val	Asp	Cys	Ile	Arg	Leu	Lys
				620					625					630

&lt;210&gt; 13

&lt;211&gt; 695

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7482007CD1

&lt;400&gt; 13

Met	Lys	Met	Lys	Ser	Gln	Ala	Thr	Met	Ile	Cys	Cys	Leu	Val	Phe
1				5					10					15
Phe	Leu	Ser	Thr	Glu	Cys	Ser	His	Tyr	Arg	Ser	Lys	Ile	His	Leu
				20					25					30
Lys	Ala	Gly	Asp	Lys	Leu	Gln	Ser	Pro	Glu	Gly	Lys	Pro	Lys	Thr
				35					40					45
Gly	Arg	Ile	Gln	Glu	Lys	Cys	Glu	Gly	Pro	Cys	Ile	Ser	Ser	Ser
				50					55					60
Asn	Cys	Ser	Gln	Pro	Cys	Ala	Lys	Asp	Phe	His	Gly	Glu	Ile	Gly
				65					70					75
Phe	Thr	Cys	Asn	Gln	Lys	Lys	Trp	Gln	Lys	Ser	Ala	Glu	Thr	Cys
				80					85					90
Thr	Ser	Leu	Ser	Val	Glu	Lys	Leu	Phe	Lys	Asp	Ser	Thr	Gly	Ala
				95					100					105
Ser	Arg	Leu	Ser	Val	Ala	Ala	Pro	Ser	Ile	Pro	Leu	His	Ile	Leu
				110					115					120
Asp	Phe	Arg	Ala	Pro	Glu	Thr	Ile	Glu	Ser	Val	Ala	Gln	Gly	Ile
				125					130					135
Arg	Lys	Asn	Cys	Pro	Phe	Asp	Tyr	Ala	Cys	Ile	Thr	Asp	Met	Val
				140					145					150
Lys	Ser	Ser	Glu	Thr	Thr	Ser	Gly	Asn	Ile	Ala	Phe	Ile	Val	Glu
				155					160					165
Leu	Leu	Lys	Asn	Ile	Ser	Thr	Asp	Leu	Ser	Asp	Asn	Val	Thr	Arg
				170					175					180
Glu	Lys	Met	Lys	Ser	Tyr	Ser	Glu	Val	Ala	Asn	His	Ile	Leu	Asp
				185					190					195
Thr	Ala	Ala	Ile	Ser	Asn	Trp	Ala	Phe	Ile	Pro	Asn	Lys	Asn	Ala
				200					205					210
Ser	Ser	Asp	Leu	Leu	Gln	Ser	Val	Asn	Leu	Phe	Ala	Arg	Gln	Leu
				215					220					225
His	Ile	His	Asn	Asn	Ser	Glu	Asn	Ile	Val	Asn	Glu	Leu	Phe	Ile
				230					235					240
Gln	Thr	Lys	Gly	Phe	His	Ile	Asn	His	Asn	Thr	Ser	Glu	Lys	Ser
				245					250					255
Leu	Asn	Phe	Ser	Met	Ser	Met	Asn	Asn	Thr	Thr	Glu	Asp	Ile	Leu
				260					265					270
Gly	Met	Val	Gln	Ile	Pro	Arg	Gln	Glu	Leu	Arg	Lys	Leu	Trp	Pro
				275					280					285
Asn	Ala	Ser	Gln	Ala	Ile	Ser	Ile	Ala	Phe	Pro	Thr	Leu	Gly	Ala
				290					295					300
Ile	Leu	Arg	Glu	Ala	His	Leu	Gln	Asn	Val	Ser	Leu	Pro	Arg	Gln
				305					310					315
Val	Asn	Gly	Leu	Val	Leu	Ser	Val	Val	Leu	Pro	Glu	Arg	Leu	Gln

	320		325		330
Glu Ile Ile Leu Thr Phe Glu Lys Ile Asn Lys Thr Arg Asn Ala					
	335		340		345
Arg Ala Gln Cys Val Gly Trp His Ser Lys Lys Arg Arg Trp Asp					
	350		355		360
Glu Lys Ala Cys Gln Met Met Leu Asp Ile Arg Asn Glu Val Lys					
	365		370		375
Cys Arg Cys Asn Tyr Thr Ser Val Val Met Ser Phe Ser Ile Leu					
	380		385		390
Met Ser Ser Lys Ser Met Thr Asp Lys Val Leu Asp Tyr Ile Thr					
	395		400		405
Cys Ile Gly Leu Ser Val Ser Ile Leu Ser Leu Val Leu Cys Leu					
	410		415		420
Ile Ile Glu Ala Thr Val Trp Ser Arg Val Val Val Thr Glu Ile					
	425		430		435
Ser Tyr Met Arg His Val Cys Ile Val Asn Ile Ala Val Ser Leu					
	440		445		450
Leu Thr Ala Asn Val Trp Phe Ile Ile Gly Ser His Phe Asn Ile					
	455		460		465
Lys Ala Gln Asp Tyr Asn Met Cys Val Ala Val Thr Phe Phe Ser					
	470		475		480
His Phe Phe Tyr Leu Ser Leu Phe Phe Trp Ile Leu Phe Lys Ala					
	485		490		495
Leu Leu Ile Ile Tyr Gly Ile Leu Val Ile Phe Arg Arg Met Met					
	500		505		510
Lys Ser Arg Met Met Val Ile Gly Phe Ala Ile Gly Tyr Gly Cys					
	515		520		525
Pro Leu Ile Ile Ala Val Thr Thr Val Ala Ile Thr Gly Pro Val					
	530		535		540
Lys Gly Tyr Met Arg Pro Glu Ala Cys Trp Leu Asn Trp Asp Asn					
	545		550		555
Thr Lys Ala Leu Leu Ala Phe Ala Ile Pro Ala Phe Val Ile Val					
	560		565		570
Ala Val Asn Leu Ile Val Val Leu Val Val Ala Val Asn Thr Gln					
	575		580		585
Arg Pro Ser Ile Gly Ser Ser Lys Ser Gln Asp Val Val Ile Ile					
	590		595		600
Met Arg Ile Ser Lys Asn Val Ala Ile Leu Thr Pro Leu Leu Gly					
	605		610		615
Leu Thr Trp Gly Phe Gly Ile Ala Thr Leu Ile Glu Gly Thr Ser					
	620		625		630
Leu Thr Phe His Ile Ile Phe Ala Leu Leu Asn Ala Phe Gln Gly					
	635		640		645
Phe Phe Ile Leu Leu Phe Gly Thr Ile Met Asp His Lys Ile Arg					
	650		655		660
Asp Ala Leu Arg Met Arg Met Ser Ser Leu Lys Gly Lys Ser Arg					
	665		670		675
Ala Ala Glu Asn Ala Ser Leu Gly Pro Thr Asn Gly Ser Lys Leu					
	680		685		690
Met Asn Arg Gln Gly					
	695				

<210> 14  
 <211> 633  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 6769042CD1

<400> 14  
 Met Tyr Phe Thr Ala Ala Ile Gly Lys His Ala Leu Leu Ser Ser

1	5	10	15
Thr Leu Pro Ser Leu Phe Met Thr Ser Thr Ala Ser Pro Val Met			
	20	25	30
Pro Thr Asp Ala Tyr His Pro Ile Ile Thr Asn Leu Thr Glu Glu			
	35	40	45
Arg Lys Thr Phe Gln Ser Pro Gly Val Ile Leu Ser Tyr Leu Gln			
	50	55	60
Asn Val Ser Leu Ser Leu Pro Ser Lys Ser Leu Ser Glu Gln Thr			
	65	70	75
Ala Leu Asn Leu Thr Lys Thr Phe Leu Lys Ala Val Gly Glu Ile			
	80	85	90
Leu Leu Leu Pro Gly Trp Ile Ala Leu Ser Glu Asp Ser Ala Val			
	95	100	105
Val Leu Ser Leu Ile Asp Thr Ile Asp Thr Val Met Gly His Val			
	110	115	120
Ser Ser Asn Leu His Gly Ser Thr Pro Gln Val Thr Val Glu Gly			
	125	130	135
Ser Ser Ala Met Ala Glu Phe Ser Val Ala Lys Ile Leu Pro Lys			
	140	145	150
Thr Val Asn Ser Ser His Tyr Arg Phe Pro Ala His Gly Gln Ser			
	155	160	165
Phe Ile Gln Ile Pro His Glu Ala Phe His Arg His Ala Trp Ser			
	170	175	180
Thr Val Val Gly Leu Leu Tyr His Ser Met His Tyr Tyr Leu Asn			
	185	190	195
Asn Ile Trp Pro Ala His Thr Lys Ile Ala Glu Ala Met His His			
	200	205	210
Gln Asp Cys Leu Leu Phe Ala Thr Ser His Leu Ile Ser Leu Glu			
	215	220	225
Val Ser Pro Pro Pro Thr Leu Ser Gln Asn Leu Ser Gly Ser Pro			
	230	235	240
Leu Ile Thr Val His Leu Lys His Arg Leu Thr Arg Lys Gln His			
	245	250	255
Ser Glu Ala Thr Asn Ser Ser Asn Arg Val Phe Val Tyr Cys Ala			
	260	265	270
Phe Leu Asp Phe Ser Ser Gly Glu Gly Val Trp Ser Asn His Gly			
	275	280	285
Cys Ala Leu Thr Arg Gly Asn Leu Thr Tyr Ser Val Cys Arg Cys			
	290	295	300
Thr His Leu Thr Asn Phe Ala Ile Leu Met Gln Val Val Pro Leu			
	305	310	315
Glu Leu Ala Arg Gly His Gln Val Ala Leu Ser Ser Ile Ser Tyr			
	320	325	330
Val Gly Cys Ser Leu Ser Val Leu Cys Leu Val Ala Thr Leu Val			
	335	340	345
Thr Phe Ala Val Leu Ser Ser Val Ser Thr Ile Arg Asn Gln Arg			
	350	355	360
Tyr His Ile His Ala Asn Leu Ser Phe Ala Val Leu Val Ala Gln			
	365	370	375
Val Leu Leu Leu Ile Ser Phe Arg Leu Glu Pro Gly Thr Thr Pro			
	380	385	390
Cys Gln Val Met Ala Val Leu Leu His Tyr Phe Phe Leu Ser Ala			
	395	400	405
Phe Ala Trp Met Leu Val Glu Gly Leu His Leu Tyr Ser Met Val			
	410	415	420
Ile Lys Val Phe Gly Ser Glu Asp Ser Lys His Arg Tyr Tyr Tyr			
	425	430	435
Gly Met Gly Trp Gly Phe Pro Leu Leu Ile Cys Ile Ile Ser Leu			
	440	445	450
Ser Phe Ala Met Asp Ser Tyr Gly Thr Ser Asn Asn Cys Trp Leu			
	455	460	465
Ser Leu Ala Ser Gly Ala Ile Trp Ala Phe Val Ala Pro Ala Leu			
	470	475	480

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Phe Val Ile Val Val Asn Ile Gly Ile Leu Ile Ala Val Thr Arg
    485                      490                      495
Val Ile Ser Gln Ile Ser Ala Asp Asn Tyr Lys Ile His Gly Asp
    500                      505                      510
Pro Ser Ala Phe Lys Leu Thr Ala Lys Ala Val Ala Val Leu Leu
    515                      520                      525
Pro Ile Leu Gly Thr Ser Trp Val Phe Gly Val Leu Ala Val Asn
    530                      535                      540
Gly Cys Ala Val Val Phe Gln Tyr Met Phe Ala Thr Leu Asn Ser
    545                      550                      555
Leu Gln Gly Leu Phe Ile Phe Leu Phe His Cys Leu Leu Asn Ser
    560                      565                      570
Glu Val Arg Ala Ala Phe Lys His Lys Ile Lys Val Trp Ser Leu
    575                      580                      585
Thr Ser Ser Ser Ala Arg Thr Ser Asn Ala Lys Pro Phe His Ser
    590                      595                      600
Asp Leu Met Asn Gly Thr Arg Pro Gly Met Ala Ser Thr Lys Leu
    605                      610                      615
Ser Pro Trp Asp Lys Ser Ser His Ser Ala His Arg Val Asp Leu
    620                      625                      630
Ser Ala Val

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<210> 15
<211> 370
<212> PRT
<213> Homo sapiens

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<220>
<221> misc_feature
<223> Incyte ID No: 7476053CD1

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<400> 15
Met Glu Ala Ala Ser Leu Ser Val Ala Thr Ala Gly Val Ala Leu
  1      5      10      15
Ala Leu Gly Pro Glu Thr Ser Ser Gly Thr Pro Ser Pro Arg Gly
    20      25      30
Ile Leu Gly Ser Thr Pro Ser Gly Ala Val Leu Pro Gly Arg Gly
    35      40      45
Pro Pro Phe Ser Val Phe Thr Val Leu Val Val Thr Leu Leu Val
    50      55      60
Leu Leu Ile Ala Ala Thr Phe Leu Trp Asn Leu Leu Val Pro Val
    65      70      75
Thr Ile Pro Arg Val Arg Ala Phe His Arg Val Pro His Asn Leu
    80      85      90
Val Ala Ser Thr Ala Val Ser Asp Glu Leu Val Ala Ala Leu Ala
    95     100     105
Met Pro Pro Ser Leu Ala Ser Glu Leu Ser Thr Gly Arg Arg Arg
   110     115     120
Leu Leu Gly Arg Ser Leu Cys His Val Trp Ile Ser Phe Asp Ala
   125     130     135
Leu Cys Cys Pro Ala Gly Leu Gly Asn Val Ala Ala Ile Ala Leu
   140     145     150
Gly Arg Asp Gly Ala Ile Thr Arg His Leu Gln His Thr Leu Arg
   155     160     165
Thr Arg Ser Arg Ala Ser Leu Leu Met Ile Ala Leu Ala Arg Val
   170     175     180
Pro Ser Ala Leu Ile Ala Leu Ala Pro Leu Leu Phe Gly Arg Gly
   185     190     195
Glu Val Cys Asp Ala Arg Leu Gln Arg Cys Gln Val Ser Arg Glu
   200     205     210
Pro Ser Tyr Ala Ala Phe Ser Thr Arg Gly Ala Phe His Leu Pro
   215     220     225

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Leu	Gly	Val	Val	Pro	Phe	Val	Tyr	Arg	Lys	Ile	Tyr	Glu	Ala	Ala	
				230					235					240	
Lys	Phe	Arg	Phe	Gly	Arg	Arg	Arg	Arg	Ala	Val	Leu	Pro	Leu	Pro	
				245					250					255	
Ala	Thr	Met	Gln	Val	Lys	Glu	Ala	Pro	Asp	Glu	Ala	Glu	Val	Val	
				260					265					270	
Phe	Thr	Ala	His	Cys	Lys	Ala	Thr	Val	Ser	Phe	Gln	Val	Ser	Gly	
				275					280					285	
Asp	Ser	Trp	Arg	Glu	Gln	Lys	Glu	Arg	Arg	Ala	Ala	Met	Met	Val	
				290					295					300	
Gly	Ile	Leu	Ile	Gly	Val	Phe	Val	Leu	Cys	Trp	Ile	Pro	Phe	Phe	
				305					310					315	
Leu	Thr	Glu	Leu	Ile	Ser	Pro	Leu	Cys	Ala	Cys	Ser	Leu	Pro	Pro	
				320					325					330	
Ile	Trp	Lys	Ser	Ile	Phe	Leu	Trp	Leu	Gly	Tyr	Ser	Asn	Ser	Phe	
				335					340					345	
Phe	Asn	Pro	Leu	Ile	Tyr	Thr	Ala	Phe	Asn	Lys	Asn	Tyr	Asn	Asn	
				350					355					360	
Ala	Phe	Lys	Ser	Leu	Phe	Thr	Lys	Gln	Arg						
				365					370						

&lt;210&gt; 16

&lt;211&gt; 324

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7480410CD1

&lt;400&gt; 16

Met	Gly	Met	Glu	Gly	Leu	Leu	Gln	Asn	Ser	Thr	Asn	Phe	Val	Leu	
1				5					10					15	
Thr	Gly	Leu	Ile	Thr	His	Pro	Ala	Phe	Pro	Gly	Leu	Leu	Phe	Ala	
				20					25					30	
Ile	Val	Phe	Ser	Ile	Phe	Val	Val	Ala	Ile	Thr	Ala	Asn	Leu	Val	
				35					40					45	
Met	Ile	Leu	Leu	Ile	His	Met	Asp	Ser	Arg	Leu	His	Thr	Pro	Met	
				50					55					60	
Tyr	Phe	Leu	Leu	Ser	Gln	Leu	Ser	Ile	Met	Asp	Thr	Ile	Tyr	Ile	
				65					70					75	
Cys	Ile	Thr	Val	Pro	Lys	Met	Leu	Gln	Asp	Leu	Leu	Ser	Lys	Asp	
				80					85					90	
Lys	Thr	Ile	Ser	Phe	Leu	Gly	Cys	Ala	Val	Gln	Ile	Phe	Leu	Tyr	
				95					100					105	
Leu	Thr	Leu	Ile	Gly	Gly	Glu	Phe	Phe	Leu	Leu	Gly	Leu	Met	Ala	
				110					115					120	
Tyr	Asp	Arg	Tyr	Val	Ala	Val	Cys	Asn	Pro	Leu	Arg	Tyr	Pro	Leu	
				125					130					135	
Leu	Met	Asn	Arg	Arg	Val	Cys	Leu	Phe	Met	Val	Val	Gly	Ser	Trp	
				140					145					150	
Val	Gly	Gly	Ser	Leu	Asp	Gly	Phe	Met	Leu	Thr	Pro	Val	Thr	Met	
				155					160					165	
Ser	Phe	Pro	Phe	Cys	Arg	Ser	Arg	Glu	Ile	Asn	His	Phe	Phe	Cys	
				170					175					180	
Glu	Ile	Pro	Ala	Val	Leu	Lys	Leu	Ser	Cys	Thr	Asp	Thr	Ser	Leu	
				185					190					195	
Tyr	Glu	Thr	Leu	Met	Tyr	Ala	Cys	Cys	Val	Leu	Met	Leu	Leu	Ile	
				200					205					210	
Pro	Leu	Ser	Val	Ile	Ser	Val	Ser	Tyr	Thr	His	Ile	Leu	Leu	Thr	
				215					220					225	
Val	His	Arg	Met	Asn	Ser	Ala	Glu	Gly	Arg	Arg	Lys	Ala	Phe	Ala	
				230					235					240	

Thr	Cys	Ser	Ser	His	Ile	Met	Val	Val	Ser	Val	Phe	Tyr	Gly	Ala	
				245						250				255	
Ala	Phe	Tyr	Thr	Asn	Val	Leu	Pro	His	Ser	Tyr	His	Thr	Pro	Glu	
				260						265				270	
Lys	Asp	Lys	Val	Val	Ser	Ala	Phe	Tyr	Thr	Ile	Leu	Thr	Pro	Met	
				275						280				285	
Leu	Asn	Pro	Leu	Ile	Tyr	Ser	Leu	Arg	Asn	Lys	Asp	Val	Ala	Ala	
				290						295				300	
Ala	Leu	Arg	Lys	Val	Leu	Gly	Arg	Cys	Gly	Ser	Ser	Gln	Ser	Ile	
				305						310				315	
Arg	Val	Ala	Thr	Val	Ile	Arg	Lys	Gly							
				320											

&lt;210&gt; 17

&lt;211&gt; 315

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 55036418CD1

&lt;400&gt; 17

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				20					25					30	
Val	Val	Met	Ala	Val	Phe	Thr	Val	Ala	Leu	Cys	Gly	Asn	Val	Leu	
				35					40					45	
Leu	Ile	Phe	Leu	Ile	Tyr	Met	Asp	Pro	His	Leu	His	Thr	Pro	Met	
				50					55					60	
Tyr	Phe	Phe	Leu	Ser	Gln	Leu	Ser	Leu	Met	Asp	Leu	Met	Leu	Val	
				65					70					75	
Cys	Thr	Asn	Val	Pro	Lys	Met	Ala	Ala	Asn	Phe	Leu	Ser	Gly	Arg	
				80					85					90	
Lys	Ser	Ile	Ser	Phe	Val	Gly	Cys	Gly	Ile	Gln	Ile	Gly	Leu	Phe	
				95					100					105	
Val	Cys	Leu	Val	Gly	Ser	Glu	Gly	Leu	Leu	Leu	Gly	Leu	Met	Ala	
				110					115					120	
Tyr	Asp	Arg	Tyr	Val	Ala	Ile	Ser	His	Pro	Leu	His	Tyr	Pro	Ile	
				125					130					135	
Leu	Met	Asn	Gln	Arg	Val	Cys	Leu	Gln	Ile	Thr	Gly	Ser	Ser	Trp	
				140					145					150	
Ala	Phe	Gly	Ile	Ile	Asp	Gly	Leu	Ile	Gln	Met	Val	Val	Val	Met	
				155					160					165	
Asn	Phe	Pro	Tyr	Cys	Gly	Leu	Arg	Lys	Val	Asn	His	Phe	Phe	Cys	
				170					175					180	
Glu	Met	Leu	Ser	Leu	Leu	Lys	Leu	Ala	Cys	Val	Asp	Thr	Ser	Leu	
				185					190					195	
Phe	Glu	Lys	Val	Ile	Phe	Ala	Cys	Cys	Val	Phe	Met	Leu	Leu	Phe	
				200					205					210	
Pro	Phe	Ser	Ile	Ile	Val	Ala	Ser	Tyr	Ala	His	Ile	Leu	Gly	Thr	
				215					220					225	
Val	Leu	Gln	Met	His	Ser	Ala	Gln	Ala	Trp	Lys	Lys	Ala	Leu	Ala	
				230					235					240	
Thr	Cys	Ser	Ser	His	Leu	Thr	Ala	Val	Thr	Leu	Phe	Tyr	Gly	Ala	
				245					250					255	
Ala	Met	Phe	Ile	Tyr	Leu	Arg	Pro	Arg	His	Tyr	Arg	Ala	Pro	Ser	
				260					265					270	
His	Asp	Lys	Val	Ala	Ser	Ile	Phe	Tyr	Thr	Val	Leu	Thr	Pro	Met	
				275					280					285	
Leu	Asn	Pro	Leu	Ile	Tyr	Ser	Leu	Arg	Asn	Arg	Glu	Val	Met	Gly	
				290					295					300	

Ala Leu Arg Lys Gly Leu Asp Arg Cys Arg Ile Gly Ser Gln His  
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<210> 18  
 <211> 324  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 7481701CD1

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 Leu Leu Phe Ile Tyr Ala Phe Ile Val Val Gly Asn Leu Val Ile  
 35 40 45  
 Ile Thr Val Val Gln Leu Asn Thr His Leu His Thr Pro Met Tyr  
 50 55 60  
 Thr Phe Ile Ser Ala Leu Ser Phe Leu Glu Ile Trp Tyr Thr Thr  
 65 70 75  
 Ala Thr Ile Pro Lys Met Leu Ser Ser Leu Leu Ser Glu Arg Ser  
 80 85 90  
 Ile Ser Phe Asn Gly Cys Leu Leu Gln Met Tyr Phe Phe His Ser  
 95 100 105  
 Thr Gly Ile Cys Glu Val Cys Leu Leu Thr Val Met Ala Phe Asp  
 110 115 120  
 His Tyr Leu Ala Ile Cys Ser Pro Leu His Tyr Pro Ser Ile Met  
 125 130 135  
 Thr Pro Lys Leu Cys Thr Gln Leu Thr Leu Ser Cys Cys Val Cys  
 140 145 150  
 Gly Phe Ile Thr Pro Val Pro Glu Ile Ala Trp Ile Ser Thr Leu  
 155 160 165  
 Pro Phe Cys Gly Ser Asn His Leu Glu His Ile Phe Cys Asp Phe  
 170 175 180  
 Leu Pro Val Leu Arg Leu Ala Cys Thr Asp Thr Arg Ala Ile Val  
 185 190 195  
 Met Ile Gln Val Val Asp Val Ile His Ala Val Glu Ile Ile Thr  
 200 205 210  
 Ala Val Met Leu Ile Phe Met Ser Tyr Asp Gly Ile Val Ala Val  
 215 220 225  
 Ile Leu Arg Ile His Ser Ala Gly Gly Arg Arg Thr Ala Phe Ser  
 230 235 240  
 Thr Cys Val Ser His Phe Ile Val Phe Ser Leu Phe Phe Gly Ser  
 245 250 255  
 Val Thr Leu Met Tyr Leu Arg Phe Ser Ala Thr Tyr Ser Leu Phe  
 260 265 270  
 Trp Asp Ile Ala Ile Ala Leu Ala Phe Ala Val Leu Ser Pro Phe  
 275 280 285  
 Phe Asn Pro Ile Ile Tyr Ser Leu Arg Asn Lys Glu Ile Lys Glu  
 290 295 300  
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 305 310 315  
 Arg Pro Gly Thr Ser Ser Lys Ile Phe  
 320

<210> 19  
 <211> 312  
 <212> PRT  
 <213> Homo sapiens



<220>  
 <221> misc\_feature  
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 Ile Ala Tyr Leu Leu Thr Leu Val Gly Asn Thr Thr Ile Ile Leu  
 35 40 45  
 Val Ser Arg Leu Asp Pro His Leu His Thr Pro Met Tyr Phe Phe  
 50 55 60  
 Leu Ala His Leu Ser Phe Leu Asp Leu Ser Phe Thr Thr Ser Ser  
 65 70 75  
 Ile Pro Gln Leu Leu Tyr Asn Leu Asn Gly Cys Asp Lys Thr Ile  
 80 85 90  
 Ser Tyr Met Gly Cys Ala Ile Gln Leu Phe Leu Phe Leu Gly Leu  
 95 100 105  
 Gly Gly Val Glu Cys Leu Leu Leu Ala Val Met Ala Tyr Asp Arg  
 110 115 120  
 Cys Val Ala Ile Cys Lys Pro Leu His Tyr Met Val Ile Met Asn  
 125 130 135  
 Pro Arg Leu Cys Arg Gly Leu Val Ser Val Thr Trp Gly Cys Gly  
 140 145 150  
 Val Ala Asn Ser Leu Ala Met Ser Pro Val Thr Leu Arg Leu Pro  
 155 160 165  
 Arg Cys Gly His His Glu Val Asp His Phe Leu Cys Glu Met Pro  
 170 175 180  
 Ala Leu Ile Arg Met Ala Cys Ile Ser Thr Val Ala Ile Asp Gly  
 185 190 195  
 Thr Val Phe Val Leu Ala Val Gly Val Val Leu Ser Pro Leu Val  
 200 205 210  
 Phe Ile Leu Leu Ser Tyr Ser Tyr Ile Val Arg Ala Val Leu Gln  
 215 220 225  
 Ile Arg Ser Ala Ser Gly Arg Gln Lys Ala Phe Gly Thr Cys Gly  
 230 235 240  
 Ser His Leu Thr Val Val Ser Leu Phe Tyr Gly Asn Ile Ile Tyr  
 245 250 255  
 Met Tyr Met Gln Pro Gly Ala Ser Ser Ser Gln Asp Gln Gly Lys  
 260 265 270  
 Phe Leu Thr Leu Phe Tyr Asn Ile Val Thr Pro Leu Leu Asn Pro  
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 305 310

<210> 20  
 <211> 1076  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 7474806CB1

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 tggaggggagc ctcccgggac atggagaagg tggacatgaa tacatcacag gaacaaggtc 180  
 tctgccagtt ctgagagaag tacaagcaag tctacctctc cctggcctac agtatcatct 240  
 ttatcctagg gctgccacta aatggcactg tcttgtggca ctctggggc caaaccaagc 300

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tctgcaagct ggtgcacttc ctgttctata tcaaccttta cggcagcatc ctgctgctga 480
cctgcatctc tgtgcaccag ttcttaggtg tgtggcacc cactgtgttcg ctgccctacc 540
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ccagtgtggc ctacaagata tggaggcctc tgggtgagtgt gagcagctgc ctcaaccag 1020
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<210> 21  
 <211> 1102  
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 <213> Homo sapiens

<220>  
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 <223> Incyte ID No: 7474840CB1

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ttttcttcaa catcttcata ttcttccagg atcagaaatc gaagcccatc gacctcatca 240
gctgtaattc ggccttcatt catgtagtga tgttcctcac tgtggtggat gcttggcctc 300
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caattgttca agtagccctg ga 1102

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<210> 22  
 <211> 2529  
 <212> DNA  
 <213> Homo sapiens

<220>  
 <221> misc\_feature  
 <223> Incyte ID No: 7475092CB1

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ctgcagcctc aggcgcgcgc gtgcccggct ccgtgcagtt ggcgctgagc gtccctgcag 180
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&lt;210&gt; 23

&lt;211&gt; 1847

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7341260CB1

&lt;400&gt; 23

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&lt;210&gt; 24

&lt;211&gt; 2031

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7473911CB1

&lt;400&gt; 24

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&lt;213&gt; Homo sapiens

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&lt;223&gt; Incyte ID No: 60203310CB1

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&lt;213&gt; Homo sapiens



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&lt;211&gt; 2322

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7482007CB1

&lt;400&gt; 32

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<212> DNA
<213> Homo sapiens

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<220>
<221> misc_feature
<223> Incyte ID No: 6769042CB1

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<212> DNA
<213> Homo sapiens

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<223> Incyte ID No: 7476053CB1

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<211> 975  
<212> DNA  
<213> Homo sapiens

<220>  
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<210> 36  
<211> 948  
<212> DNA  
<213> Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 55036418CB1

&lt;400&gt; 36

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&lt;210&gt; 37

&lt;211&gt; 1086

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7481701CB1

&lt;400&gt; 37

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&lt;210&gt; 38

&lt;211&gt; 1529

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;223&gt; Incyte ID No: 7481774CB1

&lt;400&gt; 38

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